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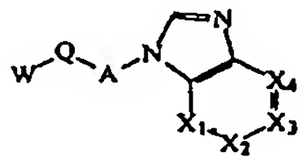

(I)

(57) Abstract: A compound of the general formula (I) or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein A represents a variety of six membered nitrogen containing heterocyclic rings, Q is a bond, halogen, C_{1-4} alkyl, O, S, SO_2, CO or CS and X_1, X_2, X_3 and X_4 are optionally substituted by 9 specific substituents or one can be nitrogen. Compositions comprising a carrier and at least one compound of formula (I) are also provided. Further provided are methods of treating tyrosine kinase-associated disease states by administering a compound of formula (I) and methods of suppressing the immune system of a subject by administering a compound of formula (I).

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I, JANENE PEISKER, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2004900103 for a patent by CYTOPIA PTY LTD OF BAKER MEDICAL RESEARCH INSTITUTE as filed on 12 January 2004.

WITNESS my hand this
First day of February 2005

JANENE PEISKER
TEAM LEADER EXAMINATION
SUPPORT AND SALES

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PROVISIONAL SPECIFICATION

Cytopia Pty Ltd

Invention Title

Selective Kinase Inhibitors

The invention is described in the following statement:

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SELECTIVE KINASE INHIBITORS

FIELD OF THE INVENTION

The present invention relates to the field of inhibitors of protein tyrosine kinases in particular the JAK family of protein tyrosine kinases.

BACKGROUND OF THE INVENTION

Protein kinases are a family of enzymes that catalyse the phosphorylation of specific residues in proteins. In general protein kinases fall into several groups; those which preferentially phosphorylate serine and/or threonine residues, those which preferentially phosphorylate tyrosine residues and those which phosphorylate both tyrosine and Ser/Thr residues. Protein kinases are therefore key elements in signal transduction pathways responsible for transducing extracellular signals, including the action of cytokines on their receptors, to the nuclei, triggering various biological events. The many roles of protein kinases in normal cell physiology include cell cycle control and cell growth, differentiation, apoptosis, cell mobility and mitogenesis.

Protein kinases include, for example, but are not limited to, members of the Protein Tyrosine Kinase family (PTKs), which in turn can be divided into the cytoplasmic PTKs and the receptor PTKs (RTKs). The cytoplasmic PTKS include the SRC family, (including: BLK; FGR; FYN; HCK; LCK; LYN; SRC;YES and YRK); the BRK Family (including: BRK; FRK, SAD; and SRM); the CSK family (including: CSK and CTK); the BTK family, (including BTK; ITK; TEC; MKK2 and TXK), the Janus kinase family, (including: JAK1, JAK2, JAK3 and Tyk2), the FAK family (including, FAK and PYK2); the Fes family (including FES and FER), the ZAP70 family (including ZAP70 and SYK); the ACK family (including ACK1 and ACK2); and the Abl family (including ABL and ARG). The RTK family includes the EGF-Receptor family (including, EGFR, HER2, HER3 and HER4); the Insulin Receptor family (including INS-R and IGF1-R); the PDGF-Receptor family (including PDGFRα, PDGFRβ, CSF1R, KIT, FLK2); the VEGF-Receptor family (including; FLT1, FLK1 and FLT4); the FGF-Receptor family (including FGFR1, FGFR2, FGFR3 and FGFR4); the CCK4 family (including CCK4); the MET family (including MET and RON); the TRK family (including TRKA, TRKB, and TRKC); the AXL family (including AXL, MER, and SKY); the TIE/TEK family (including TIE and TIE2/TEK); the

EPH family (including EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA6, EPHA7, EPHA8, EPHB1, EPHB2, EPHB3, EPHB4, EPHB5, EPHB6); the RYK family (including RYK); the MCK family (including MCK and TYRO10); the ROS family (including ROS); the RET family (including RET); the LTK family (including LTK and ALK); the ROR family (including ROR1 and ROR2); The Musk family (including Musk); the LMR family including LMR1, LMR2 and LMR3); and the SuRTK106 family (including SuRTK106).

Similarly, the serine /threonine specific kinases comprise a number of distinct sub-families, including; the extracellular signal regulated kinases, (p42/ERK2 and p44/ERK1); c-Jun NH2-terminal kinase (JNK); cAMP-responsive element-binding protein kinases (CREBK); cAMP-dependent kinase (CAPK); mitogen-activated protein kinase-activated protein kinase (MAPK and its relatives); stress-activated protein kinase p38/SAPK2; mitogen-and stress-activated kinase (MSK); protein kinases, PKA, PKB and PKC inter alia.

Additionally, the genomes of a number of pathogenic organisms possess genes encoding protein kinases. For example, the malarial parasite Plasmodium falciparum and viruses such as HPV and Hepatitis viruses appear to bear kinase related genes.

Inappropriately high protein kinase activity has been implicated in many diseases resulting from abnormal cellular function. This might arise either directly or indirectly, for example by failure of the proper control mechanisms for the kinase, related for example to mutation, over-expression or inappropriate activation of the enzyme; or by over- or under-production of cytokines or growth factors also participating in the transduction of signals upstream or downstream of the kinase. In all of these instances, selective inhibition of the action of the kinase might be expected to have a beneficial effect. Diseases where aberrant kinase activity has been implicated include: diabetes; restenosis; atherosclerosis; fibrosis of the liver and kidney; ocular diseases; myelo- and lymphoproliferative disorders; cancer such as prostate cancer, colon cancer, breast cancer, head and neck cancer, leukemia and lymphoma; and, auto-immune diseases such as Atopic Dermatitis, Asthma, rheumatoid arthritis, Crohn's disease, psoriasis, Crouzon syndrome, achondroplasia, and thanatophoric dysplasia.

The JAK family of protein tyrosine kinases (PTKs) play a central role in the cytokine dependent regulation of the proliferation and end function of several important cell types of the immune system.

A direct comparison of the four currently known mammalian JAK family members reveals the presence of seven highly conserved domains (Harpur et al, 1992). In seeking a nomenclature for the highly conserved domains characteristic of this family of PTKs, the classification used was guided by the approach of Pawson and co-workers (Sadowski et al, 1986) in their treatment of the SRC homology (SH) domains. The domains have been enumerated accordingly with most C-terminal homology domain designated JAK Homology domain 1 (JH1). The next domain N-terminal to JH1 is the kinase-related domain, designated here as the JH2 domain. Each domain is then enumerated up to the JH7 located at the N-terminus. The high degree of conservation of these JAK homology (JH) domains suggests that they are each likely to play an important role in the cellular processes in which these proteins operate. However, the boundaries of the JAK homology domains are arbitrary, and may or may not define functional domains. Nonetheless, their delineation is a useful device to aid the consideration of the overall structural similarity of this class of proteins.

The feature most characteristic of the JAK family of PTKs is the possession of two kinase-related domains (JH1 and JH2) (Wilks et al, 1991). The putative PTK domain of JAK1 (JH1) contains highly conserved motifs typical of PTK domains, including the presence of a tyrosine residue at position 1022 located 11 residues C-terminal to sub-domain VII that is considered diagnostic of membership of the tyrosine-specific class of protein kinases Alignment of the human JAK1 PTK domain (255 amino acids), with other members of the PTK class of proteins revealed homology with other functional PTKs (for example, 28% identity with c-fes (Wilks and Kurban, 1988) and 37% homology to TRK (Kozma et al, 1988)). The JH1 domains of each of the JAK family members possess an interesting idiosyncrasy within the highly conserved sub-domain VIII motif (residues 1015 to 1027 in JAK2) that is believed to lie close to the active site, and define substrate specificity. The phenylalanine and tyrosine residues flanking the conserved tryptophan in this motif are unique to the JAK family of PTKs. Aside from this element, the JH1 domains of each of the members of the JAK family are typical PTK domains. Furthermore, there is high sequence identity in the JAK family particularly in and around the ATP binding site (Figure 1).

The central role played by the JAK family of protein tyrosine kinases in the cytokine dependent regulation of the proliferation and end function of several important cell types

means that agents which inhibit JAK are useful in the prevention and chemotherapy of disease states dependent on these enzymes. Potent and specific inhibitors of each of the currently known four JAK family members will provide a means of inhibiting the action of those cytokines that drive immune pathologies, such as asthma and as immunosuppressive agents for, amongst others, organ transplants, lupus, multiple sclerosis, rheumatoid arthritis, psoriasis, Type I diabetes and complications from diabetes, cancer, atopic dermatitis, autoimmune thyroid disorders, ulcerative colitis, Crohn's disease, Alzheimer's disease, and leukemia/lymphoma.

The JAK/STAT Pathway

The delineation of a particularly elegant signal transduction pathway downstream of the non-protein tyrosine kinase cytokine receptors has recently been achieved. In this pathway the key components are: (i) A cytokine receptor chain (or chains) such as the Interleukin-4 receptor or the Interferon γ receptor; (ii) a member (or members) of the JAK family of PTKs; (iii) a member(s) of the STAT family of transcription factors, and (iv) a sequence specific DNA element to which the activated STAT will bind.

A review of the JAK/STAT literature offers strong support to the notion that this pathway is important for the recruitment and marshalling of the host immune response to environmental insults, such as viral and bacterial infection. This is well exemplified in Table 1 and Table 2. Information accumulated from gene knock-out experiments have underlined the importance of members of the JAK family to the intracellular signalling triggered by a number of important immune regulatory cytokines. The therapeutic possibilities stemming from inhibiting (or enhancing) the JAK/STAT pathway are thus largely in the sphere of immune modulation, and as such are likely to be promising drugs for the treatment of a range of pathologies in this area. In addition to the diseases listed in Tables 1 and 2, inhibitors of JAKs could be used as immunosuppresive agents for organ transplants and autoimmune diseases such as lupus, multiple sclerosis, rheumatoid arthritis, Type I diabetes, autoimmune thyroid disorders, Alzheimer's disease and other autoimmune diseases. Additionally, treatment of cancers such as prostate cancer by JAK inhibitors is indicated.

Table 1 – Activation of the JAK/STAT pathway in various pathologies

Disease Type	Cell Types Involved	Characteristics
Atopy Allergic Asthma Atopic Dermatitis (Eczema) Allergic Rhinitis	(Mast Cells (Eosinophils (T-Cells (B-Cells	T-cell activation of B-cells followed by IgE mediated activation of resident Mast cells and Eosinophils
Cell Mediated Hypersensitivity Allergic Contact Dermatitis Hypersensitivity Pneumonitis	(T-cells (B-cells	T-cell hypersensitivity
Rheumatic Diseases Systemic Lupus Erythematosus (SLE) Rheumatoid Arthritis Juvenile Arthritis Sjögren's Syndrome Scleroderma Polymyositis Ankylosing Spondylitis Psoriatic Arthritis	(Monocytes (Macrophages (Neutrophils (Mast Cells (Eosinophils (T-Cells (B-Cells	Cytokine Production (e.g.TNF, IL-1, CSF-1, GM-CSF) T-cell Activation JAK/STAT activation
Transplantation Transplant rejection	T-Cells & B-Cells	JAK/STAT Activation

Table 1 (cont.)

Disease Type	Cell Types Involved	Characteristics
Graft versus Host Disease	T-Cells & B-Cells	JAK/STAT Activation
Viral Diseases		
Epstein Barr Virus (EBV)	Lymphocytes	JAK/STAT Activation
Hepatitis B	Hepatocytes	JAK/STAT Activation
Hepatitis C	Hepatocytes	JAK/STAT Inhibition
HIV	Lymphocytes	JAK/STAT Activation
HTLV 1	Lymphocytes	JAK/STAT Activation
Varicella-Zoster Virus (VZV)	Fibroblasts	JAK/STAT Inhibition
Human Papilloma Virus (HPV)	Epithelial cells	JAK/STAT Inhibition
Cancer		
Leukemia	Leucocytes	(Cytokine production
Lymphoma	Lymphocytes	(JAK/STAT Activation

Table 2: Diseases Potentially Treatable By JAK-Based Drug Therapies

Target Disease	Cytokine	JAK family member	Strength of Association
Asthma	IL-4 & IL-9	JAK1 &JAK3	+++
	IL-13	JAK1 & JAK2	+++
	IL-5	JAK2	+++
Eczema	IL-4	JAK1 & JAK3	+++
	IFN-α	JAK1 & JAK2	+++
Food Allergy	IL-4	JAK1 & JAK3	+++
Inflammatory Bowel Disease & Crohn's Disease	IL-4	JAK1 & JAK3	+++
Leukaemia And Lymphoma	(IL-2)	JAK3, JAK1 & JAK2	+++
Transplantation			
B-Cell Maturation	IL-4	JAK1 & JAK3	+++
T-Cell Proliferation	IL-2	JAK1 & JAK3	+++
Cutaneous Inflammation	GM-CSF & IL-6	JAK1 & JAK2	+++

Table 2 (cont.)

Target Disease	Cytokine	JAK family member	Strength of Association
Immune Suppression By Solid Tumour	IL-10	JAK1 & TYK2	+++
Prostate Cancer	IL-6	JAK1, JAK2 &Tyk2	+++

Jak 3 Signalling

Although the other members of the Jak family are expressed by essentially all tissues, JAK3 expression appears to be limited to hematopoetic cells. This is consistent with its essential role in signaling through the receptors for IL-2, IL4, IL-7, IL-9 and IL-15 by non-covalent association of JAK3 with the gamma chain common to these multichain receptors. Males with X-linked severe combined immunodeficiency (XSCID) have defects in the common cytokine receptor gamma chain (gamma c) gene that encodes a shared, essential component of the receptors of interleukin-2 (IL-2), IL-4, IL-7, IL-9, and IL-15. An XSCID syndrome in which patients with either mutated or severely reduced levels of JAK3 protein has been identified, suggesting that immunosuppression should result from blocking signalling through the JAK3 pathway. Gene Knock out studies in mice have suggested that JAK3 not only plays a critical role in B and T lymphocyte maturation, but that JAK3 is constitutively required to maintain T cell function. Taken together with the biochemical evidence for the involvement of JAK3 in signalling events downstream of the IL-2 and IL-4 receptor, these human and mouse mutation studies suggest that modulation of immune activity through the inhibiton of JAK3 could prove useful in the treatment of T- cell and B-cell proliferative disorders such as transplant rejection and autoimmune diseases.

Prolonged immunomodulation through inhibition of JAK3 signalling should have great therapeutic potential as long as JAK3 inhibition was achieved selectively and not

accompanied by inhibition of other kinase-dependent signalling processes. In particular, the high degree of sequence identity held in common by members of the JAK family of kinases raises the possibility that a compound which inhibits Jak3 would also inhibit other members of the family with detrimental long term consequences. For example, prolonged inhibition of Jak2 is likely to lead to erythropenia and thrombocytopenia, since the receptors for both erythropoietin and thrombopoietin use only JAK2 for intracellular transmission of signals.

Selective and Irreversible Inhibition

A PTK catalyses the transfer of a phosphate group from a molecule of ATP to a tyrosine residue located on a protein substrate. The inhibitors known in the art are usually competitive with either the ATP or the protein substrate of the kinase (Levitzki 2000). Since the concentration of ATP in a cell is normally very high (millimolar), compounds that are competitive with ATP may lack in vivo activity since it is unlikely that said compounds can reach the concentrations within the cell that are necessary to displace the ATP from its binding site.

An alternative approach which has been attempted in relation to EGFR is to design or select compounds which bind to EGFR TK in an irreversible manner. Such compounds are disclosed in Fry 1998; Discafani 1999; Smaill 1999; Smaill 2000; Tsou 2001; Smaill 2001; Wissner 2003. These compounds function as irreversible inhibitors by virtue of the fact that they can form covalent bonds to amino acid residues located at the active site of the enzyme which results in enhanced potency of the compounds in vitro and in the inhibition of growth of human tumors in in vivo models of cancer. A further benefit of such irreversible inhibitors when compared to reversible inhibitors, is that irreversible inhibitors can be used in prolonged suppression of the tyrosine kinase, limited only by the normal rate of receptor turnover.

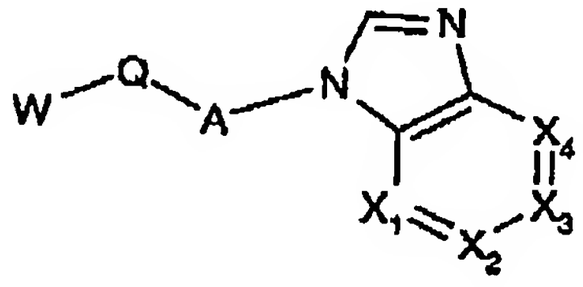
The high homology between members of the JAK family of kinases makes the design of compounds with acceptable selectivity highly challenging. It is believed that by exploiting the minor differences in the amino acid sequence between the members of this family may allow for the identification of selective inhibitors. Alignment of the four members of the JAK family of protein tyrosine kinases reveals that within the amino acids that comprise the ATP-binding pocket of these kinases there are very few amino acid

differences that could be used to target potential inhibitors towards one family member or another. Interestingly, JAK3 alone amongst this sub-family of kinases possesses a Cysteine residue close to the front lip of the ATP-binding cavity. It was hypothesised that this may provide a means to develop highly specific irreversible JAK3 inhibitors (Figure 2), by targeting this Cysteine with a functionality bearing an alkylating group such as a Michael acceptor.

SUMMARY OF THE INVENTION

The present inventors have found that a group of compounds based upon a disubstituted pyrazine scaffold which include an alkylating group such as a Michael acceptor are irreversible and selective inhibitors of the enzyme Janus Kinase 3 and as such may be useful in therapy as immunosuppressive agents for organ transplants, lupus, multiple sclerosis, rheumatoid arthritis, psoriasis, Type I diabetes and complications from diabetes, cancer, asthma, atopic dermatitis, autoimmune thyroid disorders, ulcerative colitis, Crohn's disease, Alzheimer's disease, and other indications where immunosuppression would be desirable. Furthermore, it is believed that these compounds may find application in therapeutic treatments for proliferative diseases such as Leukemia and Lymphoma where JAK3 is hyperactivated.

Accordingly, in a first aspect the present invention provides a compound of the general formula I

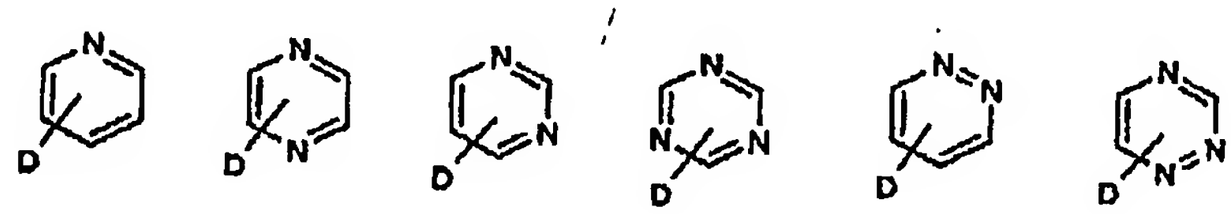


I

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

X_1, X_2, X_3, X_4 are each carbon where one is substituted with Z and the rest independently with Y; or one of X_1, X_2, X_3, X_4 is N, and the others are carbon where one carbon is substituted with Z and the rest independently with Y;

A is a ring selected from:



where D is selected from H, C_{1-4} alkyl, halogen, amino;

Q is a bond, halogen, C_{1-4} alkyl, O, S, SO, SO_2, CO, CS;

W is:

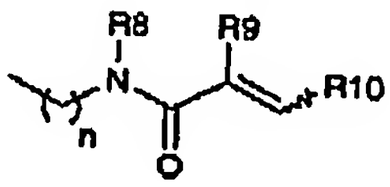
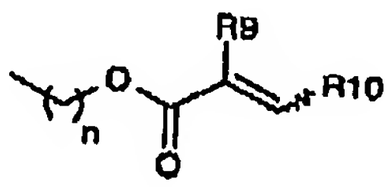
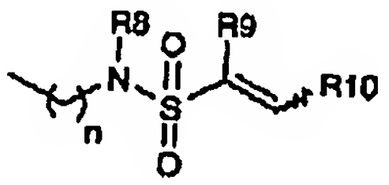
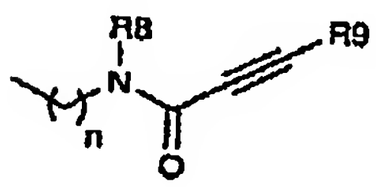
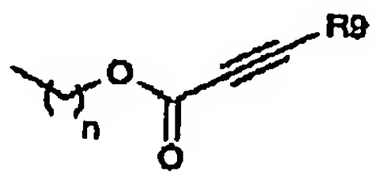
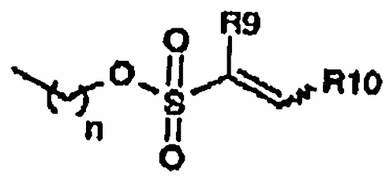
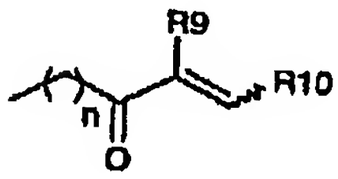
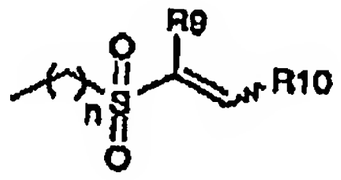
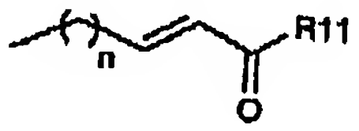
(i) NR1R2 where R1 and R2 are independently H, C_{1-4} alkyl, C_{1-4} alkylCF_3, aryl, hetaryl, C_{1-4} alkylaryl, C_{1-4} alkylhetaryl, C_{3-8} cycloalkyl, C_{2-6} alkenyl, cyclohetalkyl, C_{1-4} alkylcycloalkyl, C_{1-4} alkyl cyclohetalkyl, or R1 and R2 are joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR3; and R3 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4} alkyl aryl, C_{1-4} alkyl hetaryl, COR4 where R4 is selected from H, C_{1-4} alkyl, aryl, hetaryl;

OR

(ii) H, C_{1-4} alkyl, aryl, hetaryl, C_{3-8} cycloalkyl, cyclohetalkyl, C_{1-4} alkylaryl, C_{1-4} alkylhetaryl, C_{3-8} cycloalkyl, C_{1-4} alkylcycloalkyl, C_{1-4} alkyl cyclohetalkyl;

Y is H, halogen, CN, CF_3, nitro, OH, C_{1-4} alkyl, C_{1-4} alkylNR5R6, C_{1-4} alkylhetaryl, OC_{1-4} alkyl, OC_{2-4} alkylOC_{1-4}alkyl, OC_{1-4} alkylNR5R6, OC_{1-4} alkylhetaryl, OC_{1-4} alkylcyclohetalkyl, SC_{1-4} alkyl, SC_{2-4} alkylOC_{1-4}alkyl, SC_{1-4} alkylNR5R6, NR5R6, NR5COR6, $NR5SO_2R6$; and R5 and R6 are each independently H, C_{1-4} alkyl, or may be joined to form an optionally substituted 3-6 membered ring optionally containing an atom selected from O, S, NR7 and R7 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4} alkylaryl, C_{1-4} alkylhetaryl;

Z is selected from :

where R8 is selected from H, C_{1-4} alkyl;

R9 and R10 are independently selected from H, C_{1-4} alkyl, C_{1-4} alkylNR12R13, C_{1-4} alkylOR12, C_{1-4} alkylhetaryl or may be joined to form a 5-8 membered ring optionally containing an atom selected from O, S, SO, SO_2, NR14;

R11 is selected from OH, OC_{1-4} alkyl, NR12R13;

n is 0-4;

where R12 and R13 are independently selected from H, C_{1-4} alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR14; and R14 is selected from H, C_{1-4} alkyl.

In a second aspect the present invention consists in a composition comprising a carrier and at least one compound of the first aspect of the invention.

In a third aspect the present invention consists in a method of treating a tyrosine kinase-associated disease state, the method comprising administering a therapeutically effective amount of at least one compound of the first aspect of the invention or a therapeutically effective amount of a composition of the second aspect of the invention.

In a further aspect the present invention provides the use of the compounds of the first aspect or the compositions of the second aspect in the preparation of medicaments for the treatment of JAK3-associated disease states.

In a yet further aspect, the present invention provides for a method of suppressing the immune system of a subject, the method comprising administering a therapeutically effective amount of at least one compound of the first aspect of the invention or a therapeutically effective amount of a composition of the second aspect of the invention.

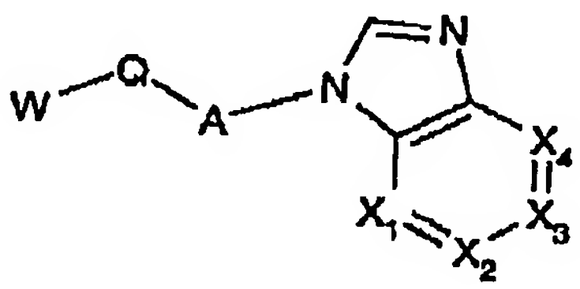
BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the amino acid sequence alignment of selected Jak Kinases

Figure 2 shows a model of the Jak3 kinase ATP binding pocket displaying the Cysteine residue.

DETAILED DESCRIPTION OF THE INVENTION

Accordingly, in a first aspect the present invention provides a compound of the general formula I

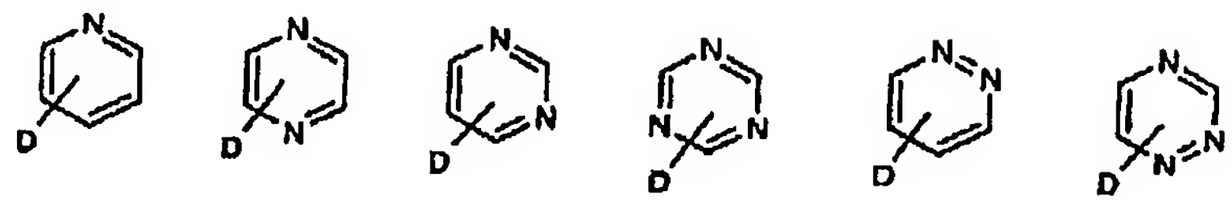


I

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

X_1, X_2, X_3, X_4 are each carbon where one is substituted with Z and the rest independently with Y; or one of X_1, X_2, X_3, X_4 is N, and the others are carbon where one carbon is substituted with Z and the rest independently with Y;

A is a ring selected from:



where D is selected from H, C_{1-4} alkyl, halogen, amino;

Q is a bond, halogen, C_{1-4} alkyl, O, S, SO, SO_2, CO, CS;

W is:

(i) NR1R2 where R1 and R2 are independently H, C_{1-4} alkyl, C_{1-4} alkylCF_3, aryl, hetaryl, C_{1-4} alkylaryl, C_{1-4} alkylhetaryl, C_{3-8} cycloalkyl, C_{2-6} alkenyl, cyclohetalkyl, C_{1-4} alkylcycloalkyl, C_{1-4} alkyl cyclohetalkyl, or R1 and R2 are joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR3; and R3 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4} alkyl aryl, C_{1-4} alkyl hetaryl, COR4 where R4 is selected from H, C_{1-4} alkyl, aryl, hetaryl;

OR

(ii) H, C_{1-4} alkyl, aryl, hetaryl, C_{3-8} cycloalkyl, cyclohetalkyl, C_{1-4} alkylaryl, C_{1-4} alkylhetaryl, C_{3-8} cycloalkyl, C_{1-4} alkylcycloalkyl, C_{1-4} alkyl cyclohetalkyl;

Y is H, halogen, CN, CF_3, nitro, OH, C_{1-4} alkyl, C_{1-4} alkylNR5R6, C_{1-4} alkylhetaryl, OC_{1-4} alkyl, OC_{2-4} alkylOC_{1-4}alkyl, OC_{1-4} alkylNR5R6, OC_{1-4} alkylhetaryl, OC_{1-4} alkylcyclohetalkyl, SC_{1-4} alkyl, SC_{2-4} alkylOC_{1-4}alkyl, SC_{1-4} alkylNR5R6, NR5R6, NR5COR6, $NR5SO_2R6$; and R5 and R6 are each independently H, C_{1-4} alkyl, or may be joined to form an optionally substituted 3-6 membered ring optionally containing an atom selected from O, S, NR7 and R7 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4} alkylaryl, C_{1-4} alkylhetaryl;

Z is selected from :

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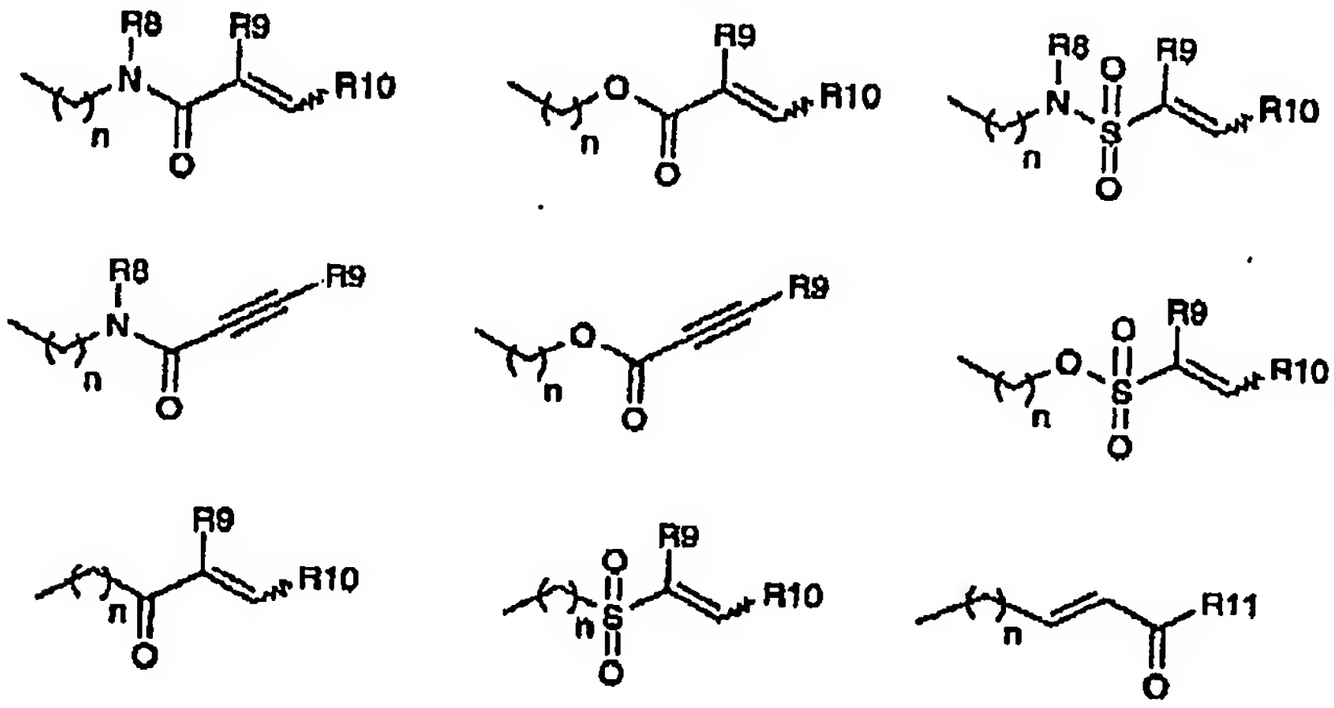
16.



where R8 is selected from H, C_{1-4} alkyl;

R9 and R10 are independently selected from H, C_{1-4} alkyl, C_{1-4} alkylNR12R13, C_{1-4} alkylOR12, C_{1-4} alkylhetaryl or may be joined to form a 5-8 membered ring optionally containing an atom selected from O, S, SO, SO_2, NR14;

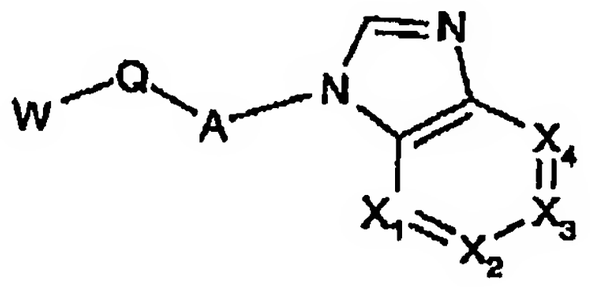
R11 is selected from OH, OC_{1-4} alkyl, NR12R13;

n is 0-4;

where R12 and R13 are independently selected from H, C_{1-4} alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR14; and R14 is selected from H, C_{1-4} alkyl.

In a preferred embodiment the compound is selected from compounds of the general formula II.

17.

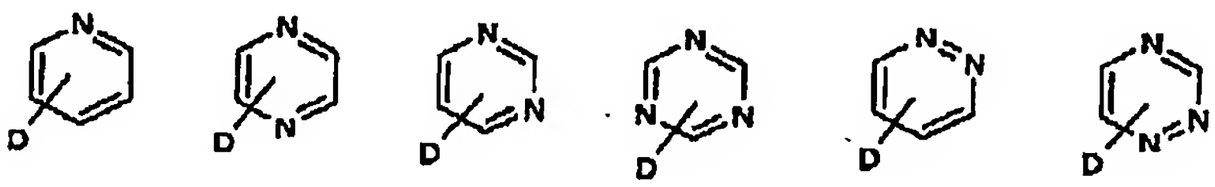


II

or pharmaceutically acceptable prodrugs, salts, hydrates, solvates, crystal forms or diastereomers thereof, wherein:

X_1, X_2, X_3, X_4 are each carbon where one is substituted with Z and the rest independently with Y; or one of X_1, X_2, X_3, X_4 is N, and the others are carbon where one carbon is substituted with Z and the rest independently with Y;

A is a ring selected from:



where D is selected from H, C_{1-4} alkyl, halogen, amino;

Q is a bond, halogen, C_{1-4} alkyl, O, S, SO, SO_2, CO, CS;

W is:

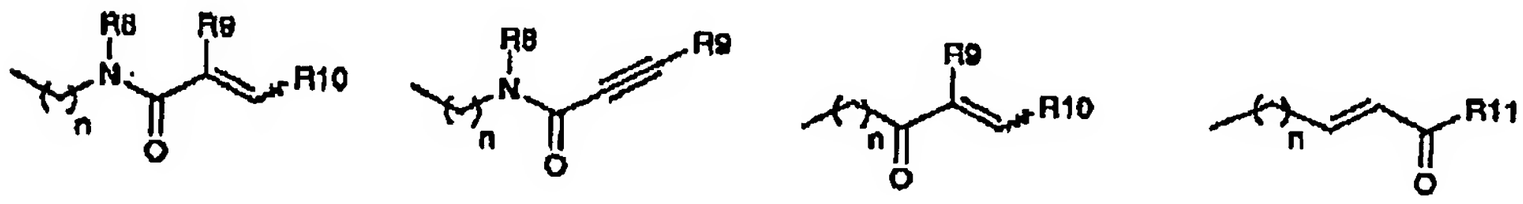
(i) NR1R2 where R1 and R2 are independently H, C_{1-4} alkyl, C_{1-4} alkylCF_3, aryl, hetaryl, C_{1-4} alkylaryl, C_{1-4} alkylhetaryl, C_{3-8} cycloalkyl, C_{2-6} alkenyl, cyclohetalkyl, C_{1-4} alkylcycloalkyl, C_{1-4} alkyl cyclohetalkyl, or R1 and R2 are joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR3; and R3 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4} alkyl aryl, C_{1-4} alkyl hetaryl, COR4 where R4 is selected from H, C_{1-4} alkyl, aryl, hetaryl;

OR

(ii) W is H, C_{1-4} alkyl, aryl, hetaryl, C_{3-8} cycloalkyl, cyclohetalkyl, C_{1-4} alkylaryl, C_{1-4} alkylhetaryl, C_{3-8} cycloalkyl, C_{1-4} alkylcycloalkyl, C_{1-4} alkyl cyclohetalkyl;

Y is H, halogen, CN, CF_3, nitro, OH, C_{1-4} alkyl, C_{1-4} alkylNR5R6, C_{1-4} alkylhetaryl, OC_{1-4} alkyl, OC_{2-4} alkylOC_{1-4}alkyl, OC_{1-4} alkylNR5R6, OC_{1-4} alkylhetaryl, OC_{1-4} alkylcyclohetalkyl, SC_{1-4} alkyl, SC_{2-4} alkylOC_{1-4}alkyl, SC_{1-4} alkylNR5R6, NR5R6, NR5COR6, $NR5SO_2R6$; and R5 and R6 are each independently H, C_{1-4} alkyl, or may be joined to form an optionally substituted 3-6 membered ring optionally containing an atom selected from O, S, NR7 and R7 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4} alkylaryl, C_{1-4} alkylhetaryl;

Z is selected from :



where R8 is selected from H, C_{1-4} alkyl;

R9 and R10 are independently selected from H, C_{1-4} alkyl, C_{1-4} alkylNR12R13, C_{1-4} alkylOR12, C_{1-4} alkylhetaryl or may be joined to form a 5-8 membered ring optionally containing an atom selected from O, S, SO, SO_2, NR14;

R11 is selected from OH, OC_{1-4} alkyl, NR12R13;

n is 0-4;

where: R12 and R13 are independently selected from H, C_{1-4} alkyl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR14; and R14 is selected from H, C_{1-4} alkyl.

In the above description it will be appreciated that:

C_{1-4} alkyl means an unsubstituted or optionally substituted straight or branched alkyl chain.

Aryl means unsubstituted or optionally substituted phenyl or naphthyl.

Hetaryl means an unsubstituted or optionally substituted 5- or 6-membered heteroaromatic ring containing one or more heteroatoms selected from O, N, S.

Cycloalkyl means a 3-8 membered saturated ring.

Cyclohetalkyl means a 3-8 membered saturated ring containing 1-3 heteroatoms selected from O, S, NR15, where R15 is H, C_{1-4} alkyl, aryl, hetaryl.

Substituents are chosen from halogen, C_{1-4} alkyl, CF_3, CN, nitro, aryl, hetaryl, OCF_3, OC_{1-4}alkyl, OC_{2-5}alkylNR16R17, Oaryl, Ohetaryl, CO_2R16, CONR16R17, nitro, NR16R17, NR16COR17, NR16SO_2R17; and R16, R17 are each independently H, C_{1-4} alkyl, C_{1-4} alkyl cycloalkyl, C_{1-4} alkyl cyclohetalkyl, aryl, hetaryl, C_{1-4}alkyl aryl, C_{1-4} alkyl hetaryl, or may be joined to form an optionally substituted 3-8 membered ring optionally containing an atom selected from O, S, NR18; and R18 is selected from H, C_{1-4} alkyl, aryl, hetaryl, C_{1-4} alkyl aryl, C_{1-4} alkyl hetaryl.

The compounds of formula I may irreversibly inhibit JAK 3. Generally, the strength of binding of reversible inhibitors of an enzyme is measured by the IC_{50} value which is a reflection of the equilibrium constant of the interaction between the inhibitor and the active site of the enzyme. Irreversible inhibitors display an apparent IC_{50} because once the inhibitor is bound it will not leave the active site and the measured IC_{50} will therefore improve (i.e. number will decrease) over time. For instance, the compound of example 20 exhibits an "IC_{50}" of ~40nM after 20 minute incubation with enzyme (prior to addition of ATP) whereas the "IC50" drops to 7nM after 90 min pre-incubation.

Preferably, the compound of formula I selectively inhibits JAK 3 with respect to JAK 1 or JAK 2. The term "selectively inhibits" is defined to mean that the apparent IC_{50} of the compound for JAK 3 is more than ten-fold lower (i.e. more potent) than the IC_{50} for JAK 1 or JAK 2.

The compounds of this invention include all conformational isomers (eg. cis and trans isomers). The compounds of the present invention have asymmetric centers and therefore exist in different enantiomeric and diastereomeric forms. This invention relates to the use of all optical isomers and stereoisomers of the compounds of the present invention, and

mixtures thereof, and to all pharmaceutical compositions and methods of treatment that may employ or contain them. The compounds of formula I may also exist as tautomers. This invention relates to the use of all such tautomers and mixtures thereof.

This invention also encompasses pharmaceutical compositions containing prodrugs of compounds of the formula I. This invention also encompasses methods of treating or preventing disorders that can be treated or prevented by the inhibition of protein kinases, such as JAK comprising administering prodrugs of compounds of the formula I. Compounds of formula I having free amino, amido, hydroxy or carboxylic groups can be converted into prodrugs. Prodrugs include compounds wherein an amino acid residue, or a polypeptide chain of two or more (eg, two, three or four) amino acid residues which are covalently joined through peptide bonds to free amino, hydroxy and carboxylic acid groups of compounds of formula I. The amino acid residues include the 20 naturally occurring amino acids commonly designated by three letter symbols and also include, 4-hydroxyproline, hydroxylysine, demosine, isodemosine, 3-methylhistidine, norvlin, beta-alanine, gamma-aminobutyric acid, citrulline, homocysteine, homoserine, ornithine and methionine sulfone. Prodrugs also include compounds wherein carbonates, carbamates, amides and alkyl esters which are covalently bonded to the above substituents of formula I through the carbonyl carbon prodrug sidechain. Prodrugs also include phosphate derivatives of compounds of formula I (such as acids, salts of acids, or esters) joined through a phosphorus-oxygen bond to a free hydroxyl of compounds of formula I.

Where the compound possesses a chiral centre the compound can be used as a purified isomer or as a mixture of any ratio of isomers. It is however preferred that the mixture comprises at least 70%, 80%, 90%, 95%, or 99% of the preferred isomer.

In a still further preferred embodiment the compound is selected from the compounds set out in Table 3.

In a second aspect the present invention consists in a composition comprising a carrier and at least one compound of the first aspect of the invention.

In a third aspect the present invention consists in a method of treating a tyrosine kinase-associated disease state, the method comprising administering a therapeutically effective

amount of at least one compound of the first aspect of the invention or a therapeutically effective amount of a composition of the second aspect of the invention.

In a further preferred embodiment the disease state involves JAK1, JAK2, JAK3 or TYK2.

In a preferred embodiment of the present invention the disease state is selected from the group consisting of Atopy, such as Allergic Asthma, Atopic Dermatitis (Eczema), and Allergic Rhinitis; Cell Mediated Hypersensitivity, such as Allergic Contact Dermatitis and Hypersensitivity Pneumonitis; Rheumatic Diseases, such as Systemic Lupus Erythematosus (SLE), Rheumatoid Arthritis, Juvenile Arthritis, Sjögren's Syndrome, Scleroderma, Polymyositis, Ankylosing Spondylitis, Psoriatic Arthritis; Other autoimmune diseases such as Type I diabetes, autoimmune thyroid disorders, and Alzheimer's disease; Viral Diseases, such as Epstein Barr Virus (EBV), Hepatitis B, Hepatitis C, HIV, HTLV 1, Varicella-Zoster Virus (VZV), Human Papilloma Virus (HPV), Cancer, such as Leukemia, Lymphoma and Prostate Cancer.

As used herein the term "tyrosine kinase-associated disease state" refers to those disorders which result from aberrant tyrosine kinase activity, in particular JAK activity and/or which are alleviated by inhibition of one or more of these enzymes.

In a further aspect the present invention provides the use of the compounds described in the preparation of medicaments for the treatment of JAK3-associated disease states.

In a yet further aspect, the present invention provides for a method of suppressing the immune system of a subject, the method comprising administering a therapeutically effective amount of at least one compound of the first aspect of the invention or a therapeutically effective amount of a composition of the second aspect of the invention.

Preferably, the method of suppressing the immune system is for the treatment of disease states selected from lupus, multiple sclerosis, rheumatoid arthritis, psoriasis, Type I diabetes and complications from diabetes, cancer, asthma, atopic dermatitis, autoimmune thyroid disorders, ulcerative colitis, Crohn's disease, and Alzheimer's disease.

Preferably, the method of suppressing the immune system is to modify the immune system response to a transplant into a subject. More preferably, the transplant is an organ transplant or tissue transplant.

The present invention provides pharmaceutical compositions comprising at least one of the compounds of the formula I or II capable of treating a JAK3-associated disorder in an amount effective therefor, and a pharmaceutically acceptable vehicle or diluent. The compositions of the present invention may contain other therapeutic agents as described below, and may be formulated, for example, by employing conventional solid or liquid vehicles or diluents, as well as pharmaceutical additives of a type appropriate to the mode of desired administration (for example, excipients, binders, preservatives, stabilizers, flavors, etc.) according to techniques such as those well known in the art of pharmaceutical formulation.

The compounds of the formula I or II may be administered by any suitable means, for example, orally, such as in the form of tablets, capsules, granules or powders; sublingually; buccally; parenterally, such as by subcutaneous, intravenous, intramuscular, or intracisternal injection or infusion techniques (e.g., as sterile injectable aqueous or non-aqueous solutions or suspensions); nasally such as by inhalation spray; topically, such as in the form of a cream or ointment; or rectally such as in the form of suppositories; in dosage unit formulations containing non-toxic, pharmaceutically acceptable vehicles or diluents. The compounds may, for example, be administered in a form suitable for immediate release or extended release. Immediate release or extended release may be achieved by the use of suitable pharmaceutical compositions comprising the present compounds, or, particularly in the case of extended release, by the use of devices such as subcutaneous implants or osmotic pumps.

In addition to primates, such as humans, a variety of other mammals can be treated according to the method of the present invention. For instance, mammals including, but not limited to, cows, sheep, goats, horses, dogs, cats, guinea pigs, rats or other bovine, ovine, equine, canine, feline, rodent or murine species can be treated. However, the method can also be practiced in other species, such as avian species (e.g., chickens).

Diseases and conditions associated with inflammation and infection can be treated using the method of the present invention. In a preferred embodiment, the disease or condition is one in which the actions of eosinophils and/or lymphocytes are to be inhibited or promoted, in order to modulate the inflammatory response.

The subjects treated in the above methods, in whom which JAK3 inhibition is desired, are mammals, including, but not limited to, cows, sheep, goats, horses, dogs, cats, guinea pigs, rats or other bovine, ovine, equine, canine, feline, rodent or murine species, and preferably a human being, male or female.

The term "therapeutically effective amount" means the amount of the subject composition that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician.

The term "composition" as used herein is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts. By "pharmaceutically acceptable" it is meant the carrier, diluent or excipient must be compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

The terms "administration of" and or "administering a" compound should be understood to mean providing a compound of the invention to the individual in need of treatment.

The pharmaceutical compositions for the administration of the compounds of this invention may conveniently be presented in dosage unit form and may be prepared by any of the methods well known in the art of pharmacy. All methods include the step of bringing the active ingredient into association with the carrier which constitutes one or more accessory ingredients. In general, the pharmaceutical compositions are prepared by uniformly and intimately bringing the active ingredient into association with a liquid carrier or a finely divided solid carrier or both, and then, if necessary, shaping the product into the desired formulation. In the pharmaceutical composition the active object compound is included in an amount sufficient to produce the desired effect upon the process or condition of diseases. As used herein, the term "composition" is intended to encompass a product comprising the specified ingredients in the specified amounts, as well as any product which results, directly or indirectly, from combination of the specified ingredients in the specified amounts.

The pharmaceutical compositions containing the active ingredient may be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily

suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Compositions intended for oral use may be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions may contain one or more agents selected from the group consisting of sweetening agents, flavoring agents, coloring agents and preserving agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients which are suitable for the manufacture of tablets. These excipients may be for example, inert diluents, such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia, and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets may be uncoated or they may be coated by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monostearate or glyceryl distearate may be employed. They may also be coated to form osmotic therapeutic tablets for control release.

Formulations for oral use may also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin, or olive oil.

Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydroxy-propylmethylcellulose, sodium alginate, polyvinyl-pyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents may be a naturally-occurring phosphatide, for example lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene

sorbitan monooleate. The aqueous suspensions may also contain one or more preservatives, for example ethyl, or n-propyl, p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

Oily suspensions may be formulated by suspending the active ingredient in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions may contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents such as those set forth above, and flavoring agents may be added to provide a palatable oral preparation. These compositions may be preserved by the addition of an anti-oxidant such as ascorbic acid.

Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents and suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, may also be present.

The pharmaceutical compositions of the invention may also be in the form of oil-in-water emulsions. The oily phase may be a vegetable oil, for example olive oil or arachis oil, or a mineral oil, for example liquid paraffin or mixtures of these. Suitable emulsifying agents may be naturally- occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions may also contain sweetening and flavoring agents.

Syrups and elixirs may be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol or sucrose. Such formulations may also contain a demulcent, a preservative and flavoring and coloring agents.

The pharmaceutical compositions may be in the form of a sterile injectable aqueous or oleagenous suspension. This suspension may be formulated according to the known art

using those suitable dispersing or wetting agents and suspending agents which have been mentioned above. The sterile injectable preparation may also be a sterile injectable solution or suspension in a non-toxic parenterally-acceptable diluent or solvent, for example as a solution in 1,3-butane diol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose any bland fixed oil may be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

The compounds of the present invention may also be administered in the form of suppositories for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient which is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

For topical use, creams, ointments, jellies, solutions or suspensions, etc., containing the compounds of the present invention are employed. (For purposes of this application, topical application shall include mouthwashes and gargles.)

The compounds of the present invention can also be administered in the form of liposomes. As is known in the art, liposomes are generally derived from phospholipids or other lipid substances. Liposomes are formed by mono- or multilamellar hydrated liquid crystals that are dispersed in an aqueous medium. Any non-toxic, physiologically acceptable and metabolisable lipid capable of forming liposomes can be used. The present compositions in liposome form can contain, in addition to a compound of the present invention, stabilisers, preservatives, excipients and the like. The preferred lipids are the phospholipids and phosphatidyl cholines, both natural and synthetic. Methods to form liposomes are known in the art.

The pharmaceutical composition and method of the present invention may further comprise other therapeutically active compounds as noted herein which are usually applied in the treatment of the above mentioned pathological conditions. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of

therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

Examples of other therapeutic agents include the following:

cyclosporins (e.g., cyclosporin A), CTLA4-Ig, antibodies such as ICAM-3, anti-IL-2 receptor (Anti-Tac), anti-CD45RB, anti-CD2, anti-CD3 (OKT-3), anti-CD4, anti-CD80, anti-CD86, agents blocking the interaction between CD40 and gp39, such as antibodies specific for CD40 and/or gp39 (i.e., CD154), fusion proteins constructed from CD40 and gp39 (CD401g and CD8gp39), inhibitors, such as nuclear translocation inhibitors, of NF-kappa B function, such as deoxyspergualin (DSG), cholesterol biosynthesis inhibitors such as HMG CoA reductase inhibitors (lovastatin and simvastatin), non-steroidal antiinflammatory drugs (NSAIDs) such as ibuprofen, aspirin, acetaminophen, leflunomide, deoxyspergualin, azathioprine and cyclooxygenase inhibitors such as rofecoxib and celecoxib, steroids such as prednisolone or dexamethasone, gold compounds, antiproliferative agents such as methotrexate, FK506 (tacrolimus, Prograf), mycophenolate mofetil, cytotoxic drugs such as azathioprine, VP-16, etoposide, fludarabine, cisplatin and cyclophosphamide, TNF-α inhibitors such as tenidap, anti-TNF antibodies or soluble TNF receptor, and rapamycin (sirolimus or Rapamune) or derivatives thereof.

When other therapeutic agents are employed in combination with the compounds of the present invention they may be used for example in amounts as noted in the Physician Desk Reference (PDR) or as otherwise determined by one of ordinary skill in the art.

In the treatment or prevention of conditions which require protein tyrosine kinase inhibition an appropriate dosage level will generally be about 0.01 to 500 mg per kg patient body weight per day which can be administered in single or multiple doses. Preferably, the dosage level will be about 0.1 to about 250 mg/kg per day; more preferably about 0.5 to about 100 mg/kg per day. A suitable dosage level may be about 0.01 to 250 mg/kg per day, about 0.05 to 100 mg/kg per day, or about 0.1 to 50 mg/kg per day. Within this range the dosage may be 0.05 to 0.5, 0..5 to 5 or 5 to 50 mg/kg per day. For oral administration, the compositions are preferably provided in the form of tablets

containing 1.0 to 1000 milligrams of the active ingredient, particularly 1.0, 5.0, 10.0, 15.0. 20.0, 25.0, 50.0, 75.0, 100.0, 150.0, 200.0, 250.0, 300.0, 400.0, 500.0, 600.0, 750.0, 800.0, 900.0, and 1000.0 milligrams of the active ingredient for the symptomatic adjustment of the dosage to the patient to be treated. The compounds may be administered on a regimen of 1 to 4 times per day, preferably once or twice per day.

It will be understood, however, that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors including the activity of the specific compound employed, the metabolic stability and length of action of that compound, the age, body weight, general health, sex, diet, mode and time of administration, rate of excretion, drug combination, the severity of the particular condition, and the host undergoing therapy.

In order that the nature of the present invention may be more clearly understood, preferred forms thereof will now be described with reference to the following non-limiting examples.

EXAMPLES

MATERIALS AND METHODS:

Compound Synthesis

Compounds of the general formula I are generally prepared from dihaloheterocycle.

When Q is a bond and W is amino, the synthesis may begin with a nucleophilic aromatic substitution to generate a monoamino-monohalo intermediate.

The nucleophilic aromatic substitution is typically carried out by addition of an amine to the di-halogenated heterocycle in a solvent such as ethanol, isopropanol, tert-butanol, dioxane, THF, DMF, toluene or xylene. The reaction is typically performed at elevated temperature in the presence of excess amine or a non-nucleophilic base such as triethylamine or diisopropylethylamine, or an inorganic base such as potassium carbonate or sodium carbonate.

Alternatively, the amino substituent may be introduced through a transition metal catalysed amination reaction. Typical catalysts for such transformations include

$Pd(OAc)_2/P(t\text{-}Bu)_3$, $Pd_2(dba)_3$/BINAP and $Pd(OAc)_2$/BINAP. These reactions are typically out in solvents such as toluene or dioxane, in the presence of bases such as caesium carbonate or sodium or potassium tert-butoxide at temperatures ranging from room temperature to reflux.

The amines employed in the first step of the synthesis of these compounds are obtained commercially or are prepared using methods well known to those skilled in the art.

When Q is a bond and W is aryl, hetaryl or other similar carbon-linked systems, the synthesis typically begins with a cross-coupling reaction between dihaloheterocycle and a suitably functionalised coupling partner. Typical coupling partners are boronic acids or esters (Suzuki coupling: see for example Miyaura and Suzuki 1995), stannanes (Stille coupling: see for example Stille 1986), Grignard reagents (Kumada coupling: Kumada, Tamao and Sumitani 1988) or organozinc species (Negishi coupling: Negishi 2002). The Suzuki coupling is the preferred coupling method and is typically performed in a solvent such as DME, THF, DMF, ethanol, propanol, toluene, or 1,4-dioxane in the presence of a base such as potassium carbonate, lithium hydroxide, caesium carbonate, sodium hydroxide, potassium fluoride or potassium phosphate. The reaction may be carried out at elevated temperatures and the palladium catalyst employed may be selected from $Pd(PPh_3)_4$, $Pd(OAc)_2$, $[PdCl_2(dppf)]$, $Pd_2(dba)_3/P(t\text{-}Bu)_3$.

The second step of the synthesis involves a nucleophilic aromatic substitution reaction of the monohalo intermediate with a benzimidazole or azabenzimidazole. The reaction is typically performed using a salt of the benzimidazole or azabenzimidazole in solvents such as THF, DMF, DMA, NMP, toluene, or xylene from room temperature to reflux. The benzimidazole or azabenzimidazole salt is prepared by reaction with a metal hydride such as sodium or potassium hydride or by reaction with caesium carbonate.

Alternatively, a metal-catalysed coupling reaction can be used to introduce the benzimidazole or azabenzimidazole ring. Typical metal catalysts include $Pd(OAc)_2$/dppf, $PdCl_2$/dppe, $Pd_2(OAc)_2/P(t\text{-}Bu)_3$, $(CuOTf)_2 \bullet PhH$. The reaction is typically performed using a base such as caesium carbonate, rubidium carbonate, potassium carbonate, sodium tert-butoxide or potassium phosphate in a solvent such as xylene, toluene, or DMF from room temperature to reflux. Auxiliary reagents such as phase transfer agents (e.g. cetrimonium bromide) or copper complexing agents (e.g. phenanthroline) may also be employed in the reaction.

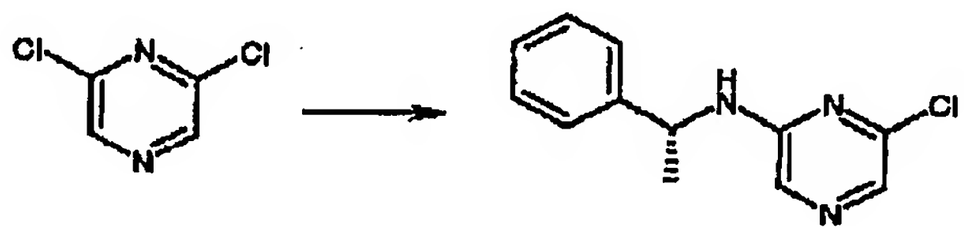
Alternatively, the reaction sequence outlined above may be reversed beginning with coupling of the benzimidazole or azabenzimidazole to the dihaloheterocycle using the methods outlined above, followed by introduction of the second substituent onto the heterocyclic nucleus using the procedures outlined above.

The products formed from either reaction sequence may be further derivatised using techniques well-known to those skilled in the art. Alternatively, derivatisation of the mono-halo intermediate may be undertaken prior to displacement of the halo substituent.

Representative syntheses are reported below.

Example 1

6-Chloro-N-[(1R)-1-phenylethyl]pyrazin-2-amine



A solution of *R*-α-methylbenzylamine (0.57g, 4.7mmol) and 2,6-dichloropyrazine (0.6388g, 4.29mmol) in dioxane (2.5 mL) was heated at reflux under N_2 for 48 hours. The solvent was removed and the product crystallised from toluene-hexane (0.82g, 82%).

^{1}H-n.m.r. ($CDCl_3$) δ 1.58 (d, *J* = 6.6Hz, 3H, CH_3), 4.88 (m, 1H, CH), 5.07 (d, 1H, NH), 7.24-7.36 (m, 5H, Ar-H), 7.61 (s, 1H, pyraz-H), 7.79 (s, 1H, pyraz-H).

Example 2

N-(tert-butyl)-6-chloropyrazin-2-amine

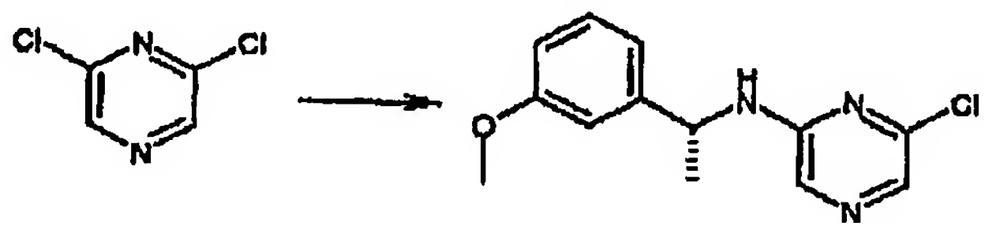
A mixture of *tert*-butylamine (14.9 g, 20 mmol), 2,6-dichloropyrazine (6.0 g, 40 mmol), Hünig's base (10mL) and ethoxyethanol (6 mL) was heated at 130°C in a sealed tube for 18

hours. The solvent was removed *In vacuo* and the residue taken up in CH_2Cl_2 (100mL) and filtered. The filtrate was washed with H_2O (2 x 20 mL), brine (20 mL) and dried (Na_2SO_4). Chromatography eluting with CH_2Cl_2 separated the product as a white solid (5.4 g, 72%).

^{1}H-n.m.r. ($CDCl_3$) δ 1.44 (s, 9H, CH_3), 4.68 (br s, 1H, NH), 7.71 (s, 1H, pyraz-H), 7.72 (s, 1H, pyraz-H).

Example 3

6-Chloro-N-[(1R)-1-(3-methoxyphenyl)ethyl]pyrazin-2-amine

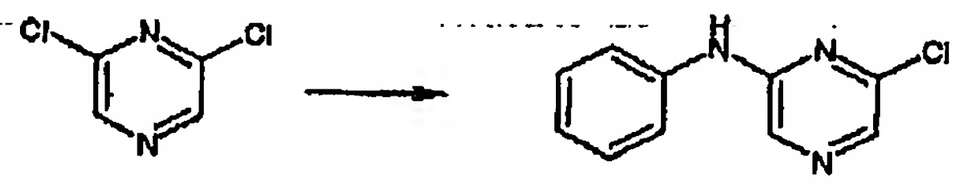


In a procedure analogous to Example 1, reaction of *R*-α-methylbenzylamine (1.0g, 6.6mmol) and 2,6-dichloropyrazine (0.440g, 2.95mmol) furnished the product (517mg, 67%).

^{1}H-n.m.r. ($CDCl_3$) δ 1.59 (d, *J*= 6.9Hz, 3H, CH_3), 3.81 (s, 3H, OCH_3), 4.87 (m, 1H, CH), 5.47 (br s, 1H, NH), 6.79-7.30 (m, 4H, Ar-H), 7.66 (s, 1H, pyraz-H), 7.79 (s, 1H, pyraz-H).

Example 4

6-Chloro-N-phenylpyrazin-2-amine



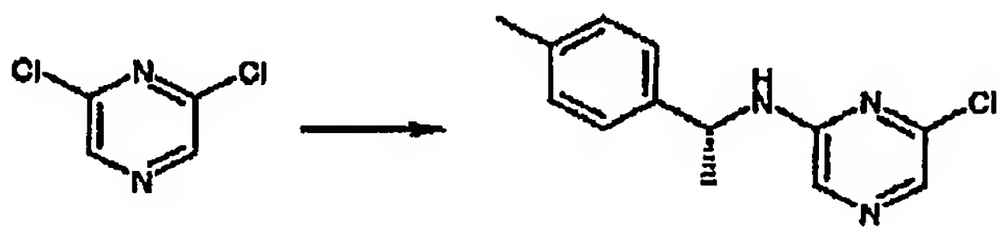
A solution of 2,6-dichloropyrazine (1 g, 6.7 mmol) and aniline (1.25 g, 13.4 mmol) in ethoxyethanol (20 mL) containing DIPEA (2.5 mL, 13.4 mmol) was heated at reflux for 3 days under N_2. The solution was concentrated under reduced pressure and the residue dissolved in EtOAc (50 mL) and washed successively with H_2O (50 mL), 1M HCl (2 x 50 mL), H_2O (50 mL) and brine (50 mL). After drying (Na_2SO_4) the solvent was removed

under reduced pressure and the residue chromatographed eluting with EtOAc-hexane (20:80 - 50:50) to separate pure product from the lower fractions (230 mg, 17%).

^{1}H-n.m.r. ($CDCl_3$) δ 6.62 (br s, 1H, NH), 7.11-7.20 (m, 1H, ArH), 7.38 (br s, 2H, ArH), 7.40 (s, 2H, ArH), 7.98 (s, 1H, pyraz-H), 8.11 (s, 1H, pyraz-H).

Example 5

6-Chloro-N-[(1R)-1-(4-methylphenyl)ethyl]pyrazin-2-amine

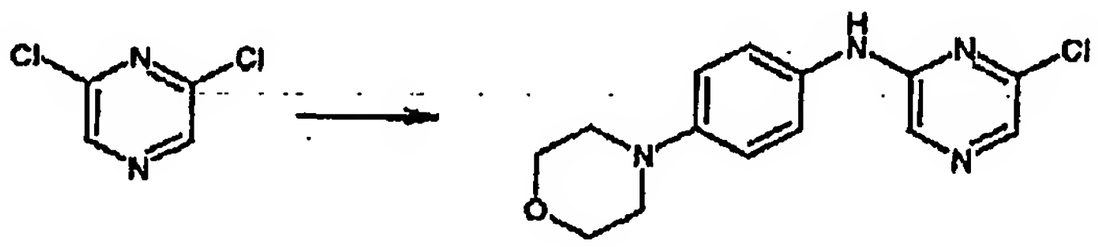


In a procedure analogous to Example 1, reaction of α-(*R*)-4-dimethylbenzylamine (250mg, 1.85mmol) and 2,6-dichloropyrazine (0.251g, 1.67mmol) furnished the product (199.5mg, 48%).

^{1}H-n.m.r. ($CDCl_3$) δ 1.56 (d, 3H, *J*= 6.9Hz, CH_3), 2.33 (s, 3H, CH_3), 4.84 (m, 1H, CH), 5.05 (br s, 1H, NH), 7.15 (AA'XX', 2H, Ar-H), 7.24 (AA'XX', 2H, Ar-H), 7.60 (s, 1H, pyraz-H), 7.78 (s, 1H, pyraz-H).

Example 6

6-Chloro-N-(4-morpholin-4-ylphenyl)pyrazin-2-amine

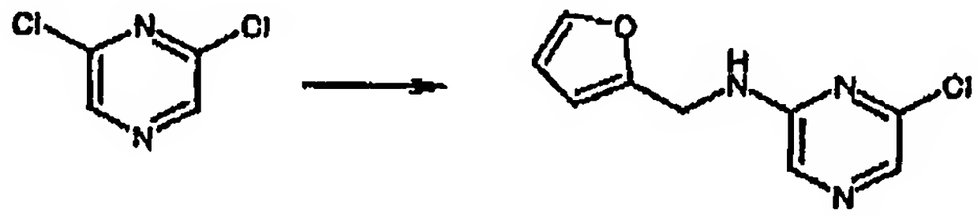


In a procedure analogous to Example 1, reaction of 4-morpholinoaniline (2.15g, 12.1mmol) and 2,6-dichloropyrazine (0.756g, 5.03mmol) furnished the product (0.54g, 37%).

^{1}H-n.m.r. ($CDCl_3$) δ3.25 (br s, 4H, CH_2), 3.99 (br s, 4H, CH_2), 7.05-7.17 (m, 2H, ArH), 7.42-7.54 (m, 2H, ArH), 7.94 (s, 1H, pyraz-H), 8.04 (s, 1H, pyraz-H), 8.06 (s, 1H, NH).

Example 7

6-Chloro-N-(2-furylmethyl)pyrazin-2-amine

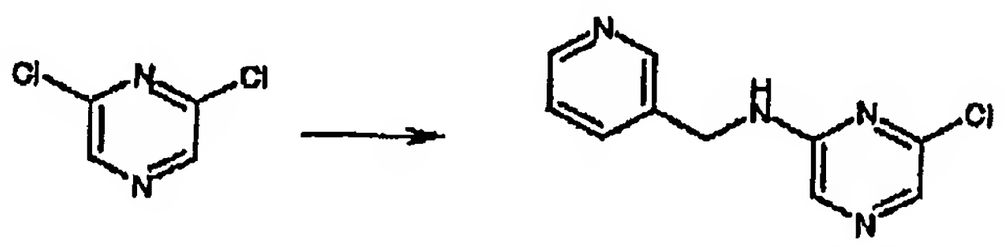


In a procedure analogous to Example 1, reaction of furfurylamine and 2,6-dichloropyrazine furnished the product (98%).

^{1}H-n.m.r. ($CDCl_3$) δ4.57 (d, J = 5.7 Hz, 2H, NCH_2), 5.01 (s, broad, 1H, NH), 6.30 (d, J = 3.3 Hz, 1H, furanyl-H), 6.35-6.33 (m, 2H, furanyl-H), 7.81 (s, 1H, pyraz.-H), 7.84 (s, 1H, pyraz.-H).

Example 8

6-Chloro-N-(pyridin-3-ylmethyl)pyrazin-2-amine

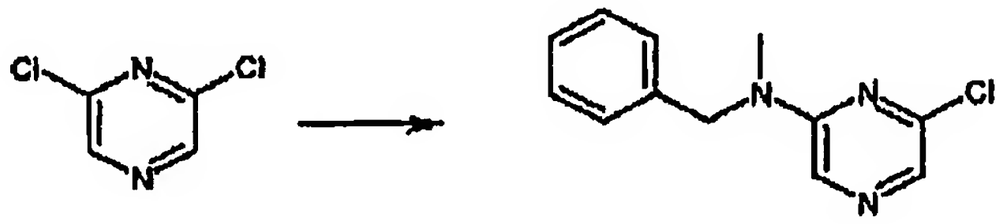


A mixture 2,6-dichloropyrazine (0.671 mmol) and 3-picolylamine (2.014 mmol) in xylene (25 ml) was refluxed overnight. The residue obtained after evaporation of the solvent was suspended between CH_2Cl_2 (100 ml) and water (100 ml). The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3 x 50 ml). The combined organic extracts were washed with brine (1 x 100 ml), dried (Na_2SO_4) and the solvent removed in vacuo. The residue was then purified by column chromatography eluting with a hexane:ethyl acetate gradient mixture to afford the desired product (93%).

^{1}H-n.m.r. ($CDCl_3$) δ4.61 (d, J = 5.7 Hz, 2 H, NCH_2), 5.29 (s, broad, 1H, NH), 7.27 (m, 1H, pyrid.-H), 7.30 (m, 1H, pyrid.-H), 7.71 (d, J = 7.8 Hz, 1H, pyrid.-H), 7.85 (s, 1H, pyrid.-H), 8.54 (s, broad, 1H, pyraz.-H), 8.61 (s, broad, 1H, pyraz.-H).

Example 9

N-Benzyl-6-chloro-N-methylpyrazin-2-amine

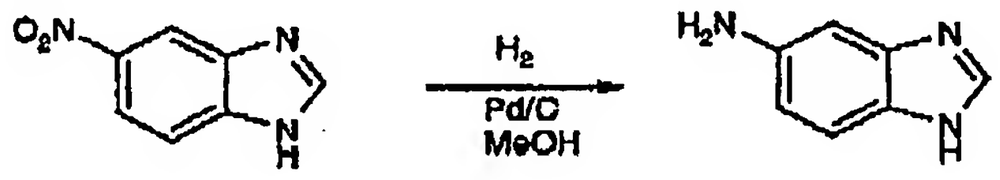


In a procedure analogous to Example 1, reaction of N-methyl benzylamine and 2,6-dichloropyrazine furnished the product (70%).

^{1}H-n.m.r. ($CDCl_3$) δ3.11 (s, 3 H, NCH_3), 4.78 (s, 2H, $ArCH_2N$), 7.24 (d, J = 6.9 Hz, 2 H, ArH), 7.37-7.28 (m, 4H, ArH), 7.81 (s, 1H, pyraz.-H), 7.88 (s, 1H, pyraz.-H).

Example 10

1H-Benzimidazol-5-amine



A solution of 5-nitrobenzimidazole (10.0 g, 61.3 mmol) in methanol (250 mL) was hydrogenated in the presence of 10% Pd/C (0.40 g) at atmospheric pressure for 20h. The mixture was filtered through Celite® and the solvent removed under reduced pressure to afford the pure product (8.1 g, 100%).

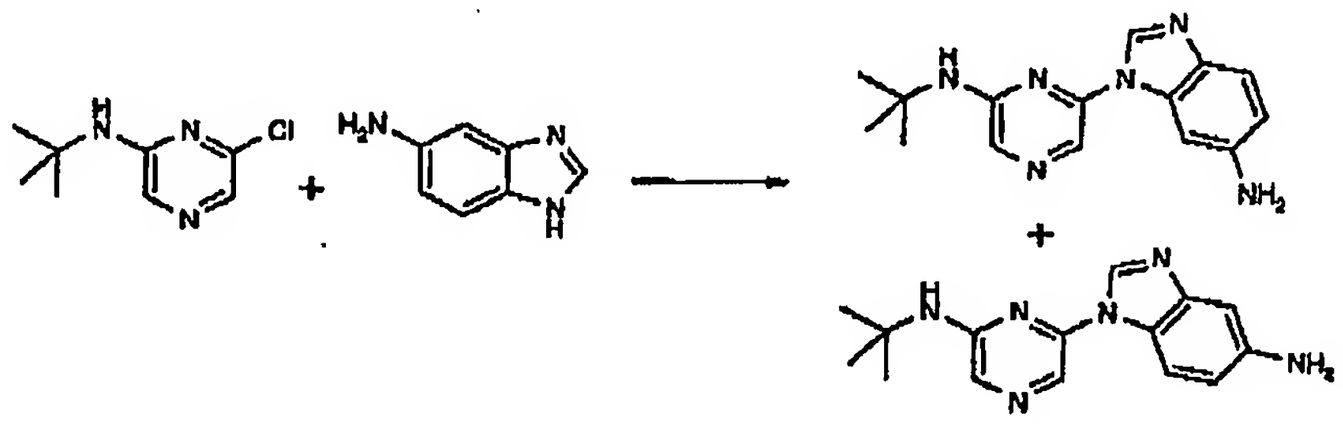
^{1}H-n.m.r. (CD_3OD) δ 6.75 (dd, 1H, J = 8.4 and 2.0 Hz, benzimid-H), 6.92 (d, 1H, J = 2.0 Hz, benzimid-H), 7.36 (d, 1H, J = 8.4 Hz, benzimid-H), 7.92 (s, 1H, benzimid-H).

Example 11

1-[6-(tert-Butylamino)pyrazin-2-yl]-1H-benzimidazol-5-amine and

1-[6-(tert-butylamino)pyrazin-2-yl]-1H-benzimidazol-6-amine

35.



A mixture of 1H-benzimidazol-5-amine (2.93 g, 22 mmol), N-(tert-butyl)-6-chloropyrazin-2-amine (3.71g, 20 mmol) and cesium carbonate (9.12 g, 28 mmol) in DMF (20 mL) was heated under N_2 for 48h. Upon cooling to RT the mixture was filtered and the filtrate concentrated *in vacuo*. The residue was extracted with $CHCl_3$ and the solvent removed under reduced pressure. The residue was chromatographed using CH_2Cl_2-MeOH (98:2 - 93:7) to give from the less polar fractions 1-[6-(*tert*-butylamino)pyrazin-2-yl]-1H-benzimidazol-6-amine (1.38 g):

^{1}H-n.m.r. ($CDCl_3$) δ 1.51 (s, 9H, CH_3), 3.80 (br s, 2H, NH_2), 4.84 (br s, 1H, NH), 6.74 (dd, 1H, J= 8.4, 2.2Hz, benzimid-H), 7.21 (d, 1H, J= 2.0Hz, benzimid-H), 7.62 (d, 1H, J= 9.2Hz, benzimid-H), 7.79 (s, 1H, pyraz-H), 8.07 (s, 1H, pyraz-H), 8.17 (s, 1H, benzimid-H).

and from the more polar fractions 1-[6-(*tert*-butylamino)pyrazin-2-yl]-1H-benzimidazol-5-amine (1.54 g):

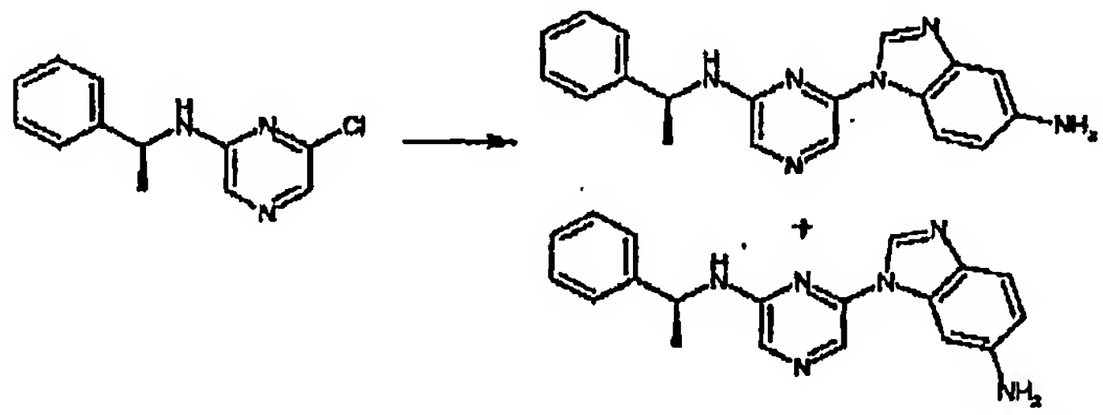
^{1}H-n.m.r. ($CDCl_3$) δ1.51 (s, 9H, CH_3), 3.48 (br s, 2H, NH_2), 4.86 (s, 1H, NH), 6.79 (dd, 1H, J = 8.6, 2.2Hz, benzimid-H), 7.14 (d, 1H, J= 2.0Hz, benzimid-H), 7.70 (d, 1H, J= 8.6Hz, benzimid-H), 7.78 (s, 1H, pyraz-H), 8.07 (s, 1H, pyraz-H), 8.47 (s, 1H, benzimid-H).

Example 12

1-(6-{[(1S)-1-Phenylethyl]amino}pyrazin-2-yl)-1H-benzimidazol-5-amine and

1-(6-{[(1S)-1-phenylethyl]amino}pyrazin-2-yl)-1H-benzimidazol-6-amine

36.



To a stirred solution of 5-amino-benzimidazole (290mg, 2.2mmol) in anhydrous DMF (10mL) under N_2 was added caesium carbonate (980mg) The resulting mixture was stirred at 70°C for 60 min. To this was added a solution of 6-chloro-N-[(1S)-1-phenylethyl]pyrazin-2-amine (470mg) in DMF (5mL) and the resulting mixture was then heated at reflux for 48h. The DMF was removed under reduced pressure and the residue diluted with chloroform. The organic layer was washed with aqueous Na_2CO_3, dried (Na_2SO_4) and the solvent removed under reduced pressure to furnish the crude product. Column chromatography using dichloromethane-methanol (95:5 - 92:8) as eluant separated two fractions from unreacted starting material. The higher Rf fraction was assigned as the 6-isomer (276mg, 42%).

^{1}H-n.m.r. ($CDCl_3$) δ1.64 (d, 3H, J= 6.9Hz, CH_3), 2.90 (br s, 2H, NH_2), 5.05 (m, 1H, CH), 5.21 (d, 1H, NH), 6.70 (dd, 1H, J= 8.7, 2.1Hz, benzimid-H), 6.97 (d, 1H, J= 1.8Hz, benzimid-H), 7.28-7.43 (m, 5H, Ph-H), 7.58 (d, 1H, J= 8.4Hz, benzimid-H), 7.84 (s, 1H, pyraz-H), 8.08 (s, 1H, pyraz-H), 8.21 (s, 1H, benzimid-H). m/z (ES) 331 (M^++H).

The lower fraction was assigned as the 5-isomer (170mg, 26%), ^{1}H-n.m.r. ($CDCl_3$) δ1.64 (d, 3H, J= 6.9Hz, CH_3), 2.85 (br s, 2H, NH_2), 5.01 (m, 1H, CH), 5. 19 (d, 1H, NH), 6.70 (dd, 1H, J= 8.7, 2.1Hz, benzimid-H), 7.11 (d, 1H, J= 1.8Hz, benzimid-H), 7.29-7.40 (m, 5H, Ph-H), 7.51 (d, 1H, J= 8.7Hz, benzimid-H), 7.81 (s, 1H, pyraz-H), 8.10 (s, 1H, pyraz-H), 8.32 (s, 1H, benzimid-H).

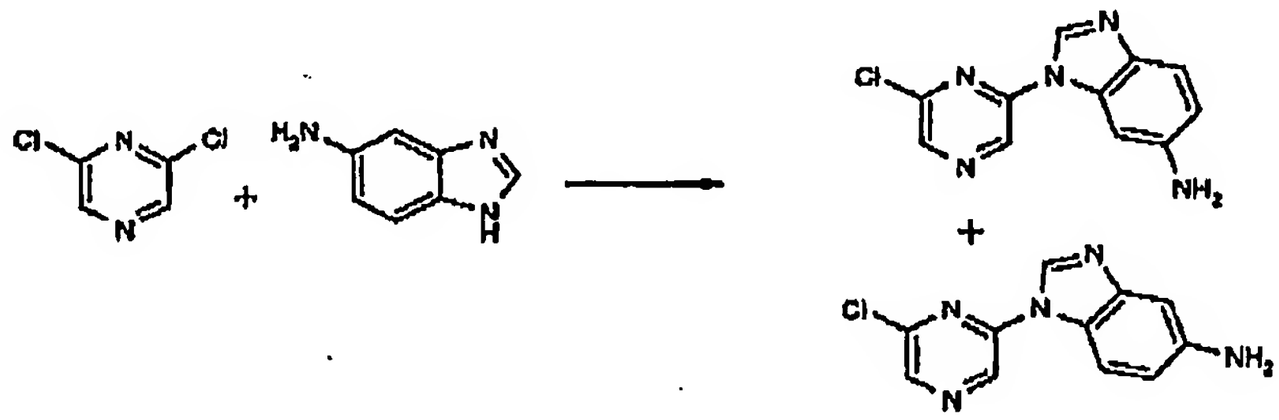
m/z (ES) 331 (M^++H).

Example 13

1-(6-Chloropyrazin-2-yl)-1H-benzimidazol-5-amine and

37.

1-(6-chloropyrazin-2-yl)-1H-benzimidazol-6-amine



A mixture of 1H-benzimidazol-5-amine (0.8 g, 6 mmol), 2,6-dichloropyrazine (0.9 g, 6.0 mmol) and cesium carbonate (2.73 g, 8.4 mmol) in DMF (6 mL) was heated under N_2 for 6h. Upon cooling to RT the mixture was diluted with dichloromethane-methanol (6:1, 30mL) and filtered and the filtrate concentrated *in vacuo*. The residue was chromatographed using CH_2Cl_2-MeOH (98:2 - 94:6) to give from the less polar fractions 1-(6-chloropyrazin-2-yl)-1H-benzimidazol-6-amine (398 mg):

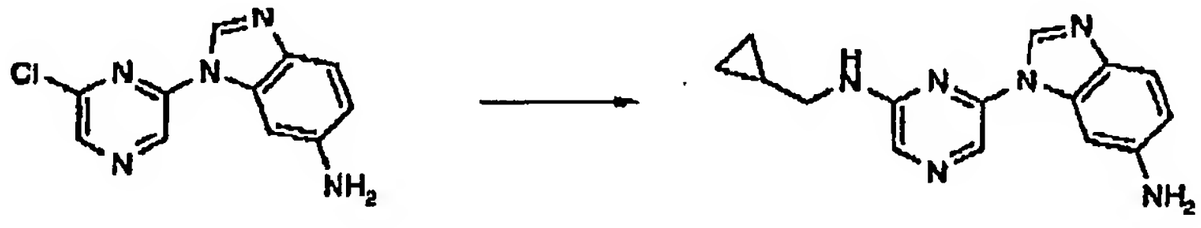
^{1}H-n.m.r. ($CDCl_3$) δ 6.74 (dd, 1H, *J*= 8.2, 2.2Hz, benzimid-H), 7.40 (d, 1H, *J*= 2.2Hz, benzimid-H), 7.51 (d, 1H, *J*= 8.2Hz, benzimid-H), 8.40 (s, 1H, pyraz-H), 8.48 (s, 1H, pyraz-H), 8.83 (s, 1H, benzimid-H).

and from the more polar fractions 1-(6-chloropyrazin-2-yl)-1H-benzimidazol-5-amine (435 mg)

^{1}H-n.m.r. ($CDCl_3$) δ6.79 (dd, 1H, *J*= 8.8, 2.2Hz, benzimid-H), 7.03 (d, 1H, *J*= 2.2Hz, benzimid-H), 7.86 (d, 1H, *J*= 9.0 Hz, benzimid-H), 8.44 (s, 1H, pyraz-H), 8.52 (s, 1H, pyraz-H), 8.82 (s, 1H, benzimid-H).

Example 14

1-[6-[(Cyclopropylmethyl)amino]pyrazin-2-yl]-1H-benzimidazol-6-amine



A solution of 1-(6-chloropytazin-2-yl)-1H-benzimidazol-6-amine (100 mg, 0.41 mmol) and cyclopropylmethylamine (424 µL, 4.1 mmol) in ethoxyethanol (2 mL) containing DIPEA (140 µL) was heated at reflux overnight under N_2. The solution was concentrated under reduced pressure and the residue dissolved in EtOAc (20 mL) and washed successively with H_2O (20 mL), 1M HCl (2 x 20 mL), H_2O (20 mL) and brine (20 mL). After drying (Na_2SO_4) the solvent was removed under reduced pressure and the residue chromatographed eluting with dichloromethane-methanol (9:1 - 94:6) to separate pure product from the lower fractions (98 mg)

^{1}H-n.m.r. ($CDCl_3$) δ0.28-0.36 (m, 2H, CH_2), 0.57-0.66 (m, 2H, CH_2), 1.08-1.22 (m, 1H, CH), 3.27-3.34 (m, 2H, CH_2), 3.79 (br s, 2H, NH_2), 5.02 (m, 1H, NH), 6.74 (dd, 1H, *J*= 8.6, 2.2Hz, benzimid-H), 7.33 (d, 1H, *J*= 2.2Hz, benzimid-H), 7.61 (d, 1H, J = 9.2Hz, benzimid-H), 7.84 (s, 1H, pyraz-H), 8.10 (s, 1H, pyraz-H), 8.35 (s, 1H, benzimid-H).

Example 15

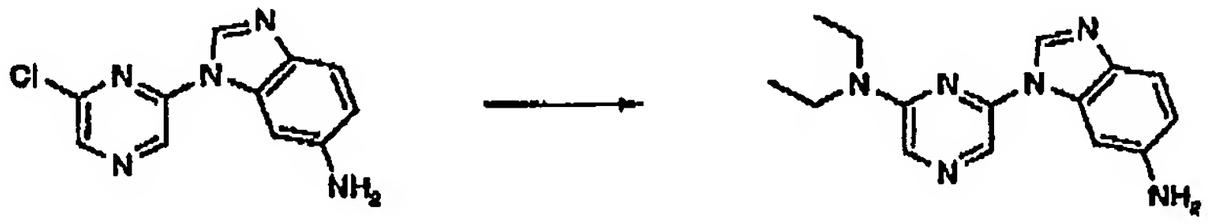
1-[6-(Isopropylamino)pyrazin-2-yl]-1H-benzimidazol-6-amine

A solution of 1-(6-chloropyrazin-2-yl)-1H-benzimidazol-6-amine (100 mg, 0.41 mmol) and isopropylamine (350 µL, 4.1 mmol) in ethoxyethanol (2 mL) containing DIPEA (140 µL) was heated in a sealed tube overnight under N_2. The solution was concentrated under reduced pressure and the residue dissolved in EtOAc (20 mL) and washed successively with H_2O (20 mL) and brine (20 mL). After drying (Na_2SO_4) the solvent was removed under reduced pressure and the residue chromatographed eluting with dichloromethane-methanol (9:1 - 94:6) to separate pure product from the lower fractions (102 mg).

^{1}H-n.m.r. ($CDCl_3$) δ1.33 (d, 6H, *J*= 6.4Hz, CH_3), 3.79 (br s, 2H, NH_2), 4.05-4.21 (m, 1H, CH), 4.72 (m, 1H, *J*= 7.2Hz, NH), 6.75 (dd, 1H, *J*= 8.6, 2.2Hz, benzimid-H), 7.32 (d, 1H, *J*= 2.0Hz, benzimid-H), 7.61 (d, 1H, *J*= 8.4Hz, benzimid-H), 7.79 (s, 1H, pyraz-H), 8.09 (s, 1H, pyraz-H), 8.35 (s, 1H, benzimid-H).

Example 16

1-[6-(Diethylamino)pyrazin-2-yl]-1H-benzimidazol-6-amine



A solution of 1-(6-chloropyrazin-2-yl)-1H-benzimidazol-6-amine (100 mg, 0.41 mmol) and diethylamine (430 µL, 4.1 mmol) in ethoxyethanol (2 mL) containing DIPEA (140 µL) was heated in a sealed tube overnight under N_2. The solution was concentrated under reduced pressure and the residue dissolved in EtOAc (20 mL) and washed successively with H_2O (20 mL) and brine (20 mL). After drying (Na_2SO_4) the solvent was removed under reduced pressure and the residue chromatographed eluting with dichloromethane-methanol (9:1 - 94:6) to separate pure product from the lower fractions (110 mg).

^{1}H-n.m.r. ($CDCl_3$) δ1.28 (t, 6H, *J*= 7.1Hz, CH_3), 3.61 (q, 4H, *J*= 7.1Hz, CH_2), 3.78 (br s, 2H, NH_2), 6.74 (dd, 1H, *J*= 8.6, 2.2Hz, benzimid-H), 7.32 (d, 1H, *J*= 2.4Hz, benzimid-H), 7.61 (d, 1H, *J*= 8.8Hz, benzimid-H), 7.91 (s, 1H, pyraz-H), 8.06 (s, 1H, pyraz-H), 8.36 (s, 1H, benzimid-H).

Example 17

1-(6-Pyridin-4-ylpyrazin-2-yl)-1H-benzimidazol-6-amine

Under a nitrogen atmosphere a mixture of 1-(6-chloropyrazin-2-yl)-1H-benzimidazol-6-amine (50 mg, 0.20 mmol), 4-pyridylboronic acid (30 mg, 0.24 mmol), tetrakis(triphenylphosphine)palladium(0) (23 mg, 0.02 mmol) in toluene—n-propanol (2 mL, 3:1) was treated with 2M aqueous sodium carbonate solution (0.14 mL, 0.84 mmol). The resulting mixture was stirred vigorously whilst being heated under reflux overnight.

Upon cooling, the mixture was diluted with ethyl acetate (10 mL) and washed with H_2O (1 x 10 mL). The aqueous phase was extracted with ethyl acetate (10 mL) and the organic layers combined and washed with 0.5M Na_2CO_3, brine and then dried (Na_2SO_4). Removal of solvent *in vacuo* then yielded crude product, which was purified by column chromatography using dichloromethane-methanol (98:2 - 91:9) as eluent to furnish the product (32 mg).

^{1}H-n.m.r. ($CDCl_3$) δ 3.88 (s, broad, 2H, NH^3), 6.80 (dd, 1H, J = 8.6 and 2.0 Hz, benzimid-H), 7.46 (d, 1H, J = 2.0 Hz, benzimid-H), 7.67 (d, 1H, J = 8.6 Hz, benzimid-H), 7.98 - 8.01 (m, 2H, pyrid-H), 8.49 (s, 1H, pyraz-H), 8.84 - 8.87 (m, 2H, pyrid-H), 8.99 (s, 1H, pyraz-H), 9.05 (s, 1H, benzimid-H).

Example 18

N-[1-[6-(tert-Butylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl]prop-2-ynamide

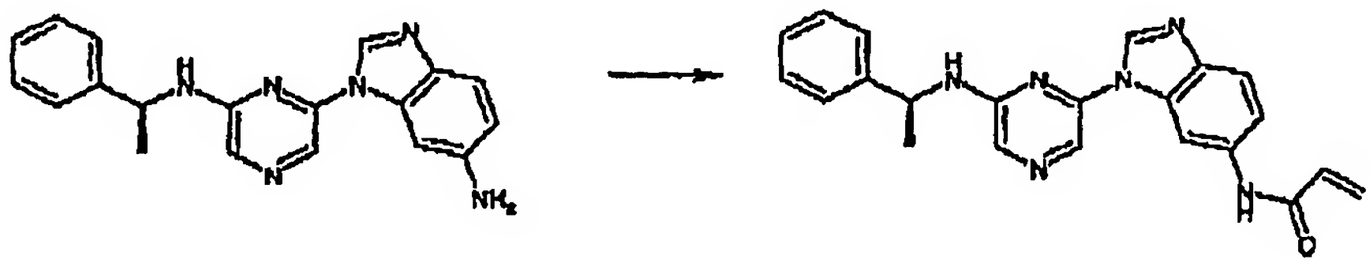
To a stirred solution of 1-[6-(tert-butylamino)pyrazin-2-yl]-1H-benzimidazol-6-amine (70 mg, 0.25 mmol) in anhydrous dichloromethane (2.5 mL) under N_2 was added triethylamine (86 µl), EDAC.HCl (60 mg), 4-(1-pyrrolidino)pyridine (4 mg) and propiolic acid (18.5 µL). The resulting mixture was then stirred at RT overnight and was the diluted with CH_2Cl_2 (10mL) and washed with H_2O (2 x 10 mL), 0.5M Na_2CO_3 (10 mL) and dried (Na_2SO_4). The solvent was removed under reduced pressure and the residue was purified by column chromatography using dichloromethane-methanol (99:1 - 91:9) as eluant to separate the pure product (1.8 mg).

^{1}H-n.m.r. ($CDCl_3$) δ1.52 (s, 9H, CH_3), 4.76 (br s, 1H, NH), 5.78 (br s, 1H, CH), 6.75 (dd, 1H, J = 8.4, 2.2 Hz, ArH), 7.22 (d, 1H, J = 2.2Hz, ArH), 7.63 (d, 1H, J = 8.0Hz, Ar-H), 7.79 (s, 1H, pyraz-H), 8.08 (s, 1H, pyraz-H), 8.37 (s, 1H, benzimid-H).

Example 19

41.

N-[1-(6-[[(1S)-1-Phenylethyl]amino]pyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide



To a stirred solution of 1-(6-[[(1S)-1-phenylethyl]amino]pyrazin-2-yl)-1H-benzimidazol-6-amine (67 mg, 0.2 mmol) in anhydrous THF (2mL) under N_2 was added triethylamine (67 µl, 0.48 mmol), EDAC.HCl (46 mg, 0.24 mmol), 4-(1-pyrrolidino)pyridine (cat.) and acrylic acid (17 mg, 0.24 mmol). The resulting mixture then stirred at RT overnight and was the diluted with H_2O (10 mL) and the mixture extracted with EtOAc (2 x 10 mL). The combined organic layers were washed with saturated aqueous Na_2CO_3, dried (Na_2SO_4) and the solvent removed in vacuo. The residue was purified by column chromatography using dichloromethane-methanol (98:2 - 94:6) as eluant to separate the pure product (25 mg).

^{1}H-n.m.r. ($CDCl_3$) δ1.62 (d, 3H, J = 6.8Hz, CH_3), 5.01-5.13 (m, 1H, CH), 5.38 (d, 1H, *J* = 6.4Hz, NH), 5.78 (dd, 1H, *J* = 9.8, 2.0Hz, CH), 6.24-6.52 (m, 2H, 2 x CH), 7.29-7.44 (m, 6H, ArH), 7.70-7.74 (m, 2H, Ar-H), 7.82 (s, 1H, pyraz-H), 8.11 (s, 1H, pyraz-H), 8.33 (s, 1H, benzimid-H), 8.42 (s, 1H, CONH).

Example 20

N-[1-[6-(tert-Butylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl]acrylamide

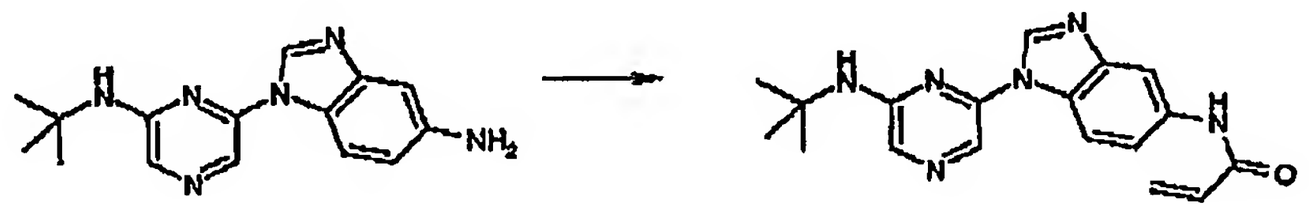
To a stirred solution of 1-[6-(tert-butylamino)pyrazin-2-yl]-1H-benzimidazol-6-amine (22 mg, 0.08 mmol) in anhydrous dichloromethane (2mL) under N_2 was added triethylamine (33 µL, 0.24 mmol), EDAC.HCl (22 mg, 0.12 mmol), 4-(1-pyrrolidino)pyridine (cat.) and acrylic acid (8 µL, 0.12 mmol). The resulting mixture then stirred at RT for 3 days and was

the diluted with H_2O (10 mL), the organic phase separated and the aqueous phase extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried (Na_2SO_4) and the solvent removed *in vacuo*. The residue was purified by column chromatography using dichloromethane-methanol (98:2 - 93:7) as eluant to separate the pure product (10 mg).

^{1}H-n.m.r. ($CDCl_3$) δ1.50 (s, 9H, CH_3), 4.89 (br s, 1H, NH), 5.77 (dd, 1H, *J*= 10.0, 2.0Hz, CH), 6.24-6.51 (m, 2H, 2 x CH), 7.25 (dd, 1H, *J*= 8.6, 2.0Hz, ArH), 7.76 (d, 1H, *J*= 8.8Hz, Ar-H), 7.83 (s, 1H, pyraz-H), 7.88 (br s, 1H, CONH), 8.13 (s, 1H, pyraz-H), 8.52 (s, 1H, benzimid-H), 8.56 (s, 1H, ArH).

Example 21

N-[1-[6-(tert-Butylamino)pyrazin-2-yl]-1H-benzimidazol-5-yl]acrylamide



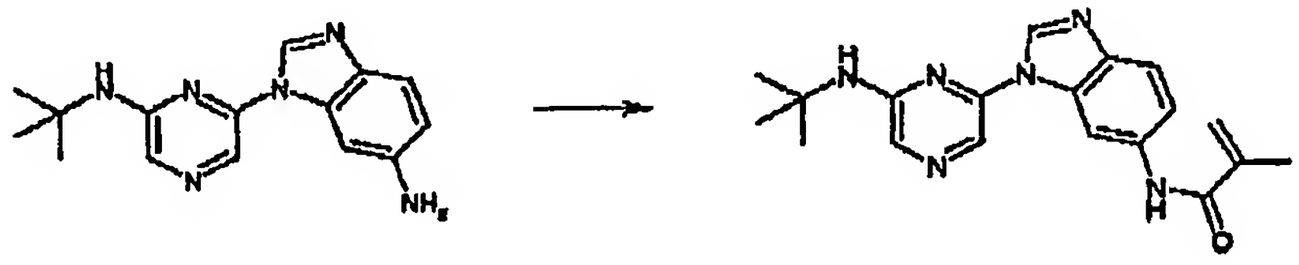
To a stirred solution of 1-[6-(tert-butylamino)pyrazin-2-yl]-1H-benzimidazol-5-amine (20 mg, 0.08 mmol) in anhydrous dichloromethane (2 mL) under N_2 was added triethylamine (33 µL, 0.24 mmol), EDAC.HCl (22 mg, 0.12 mmol), 4-(1-pyrrolidino)pyridine (cat.) and acrylic acid (8 µL, 0.12 mmol). The resulting mixture then stirred at RT for 3 days and was the diluted with H_2O (10 mL), the organic phase separated and the aqueous phase extracted with CH_2Cl_2 (10 mL). The combined organic layers were dried (Na_2SO_4) and the solvent removed *in vacuo*. The residue was purified by column chromatography using dichloromethane-methanol (98:2 - 92:8) as eluant to separate the pure product (10 mg).

^{1}H-n.m.r. ($CDCl_3$) δ1.52 (s, 9H, CH_3), 4.87 (br s, 1H, NH), 5.77 (dd, 1H, *J*= 9.8, 2.0Hz, CH), 6.31 (dd, 1H, *J*= 16.6, 9.8Hz, =CH(H)), 6.48 (dd, 1H, *J*= 16.6, 2.0Hz, =CH(H)), 7.73-7.81 (m, 2H, pyraz-H + ArH), 7.89 (d, 1H, *J*= 8.8Hz, ArH), 8.01 (s, 1H, ArH), 8.10 (s, 1H, pyraz-H), 8.55 (s, 1H, benzimid-H).

Example 22

N-[1-[6-(tert-Butylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl]-2-methylacrylamide

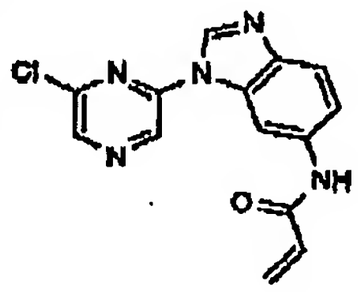
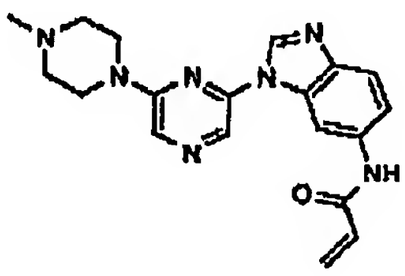
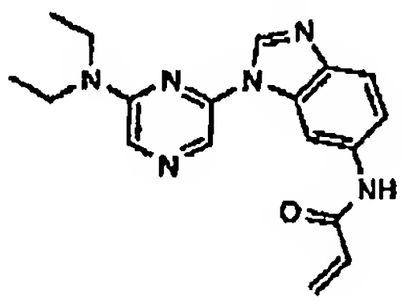
43.

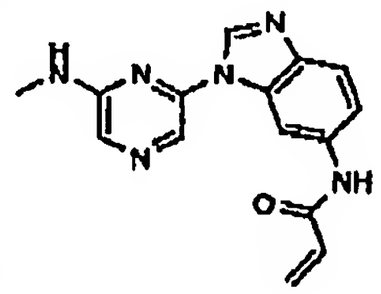
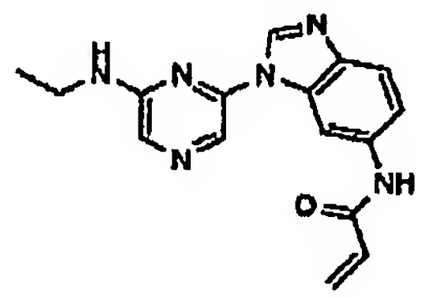
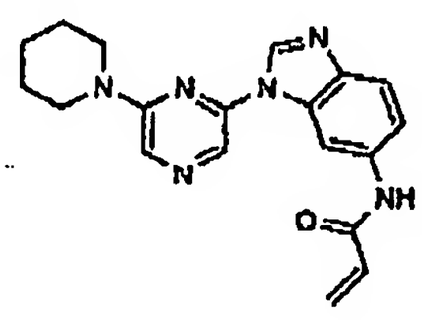


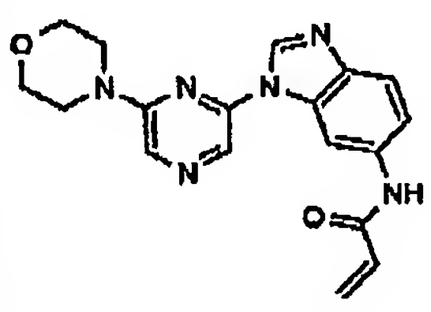
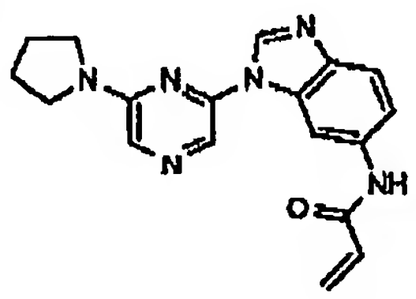
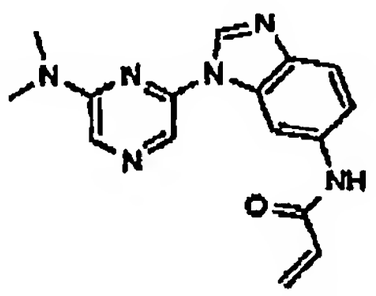
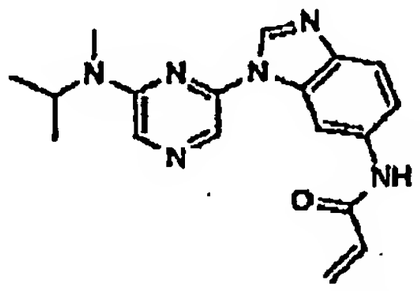
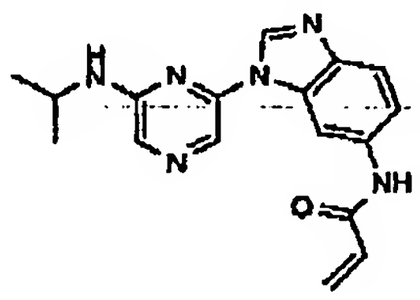
Following a procedure identical to Example 21 however using methacrylic acid in place of acrylic acid, 1-[6-(tert-butylamino)pyrazin-2-yl]-1H-benzimidazol-5-amine (57 mg) afforded N-{1-[6-(tert-butylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl}-2-methylacrylamide (54mg).

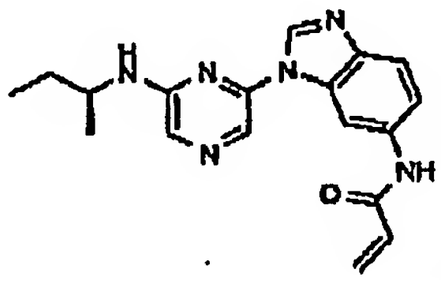
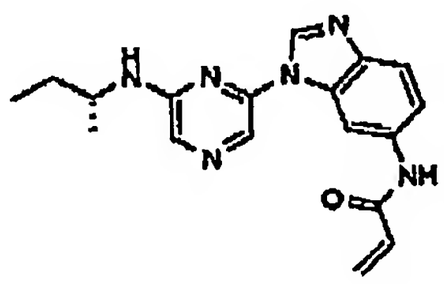
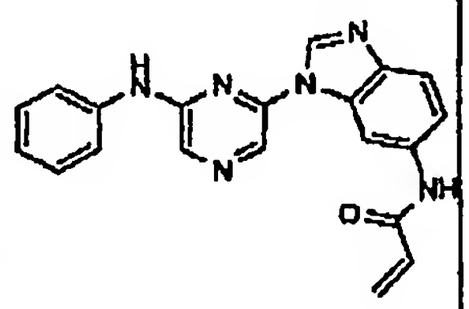
^{1}H-n.m.r. ($CDCl_3$+ d_4-MeOD) δ1.43 (s, 9H, CH_3), 2.00 (br s, 3H, CH_3), 5.42 (br s, 1H, =CH(H)), 5.77 (br s, 1H, =CH(H)), 7.32 (dd, 1H, J= 8.2, 2.0 Hz, ArH), 7.67 (d, 1H, J= 8.8 Hz, ArH), 7.74 (s, 1H, pyraz-H), 7.99 (s, 1H, pyraz-H), 8.38 (d, 1H, J= 2.0 Hz, ArH), 8.46 (s, 1H, benzimid-H).

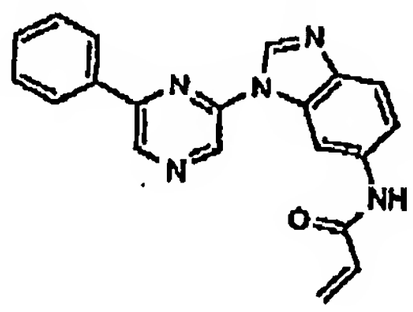
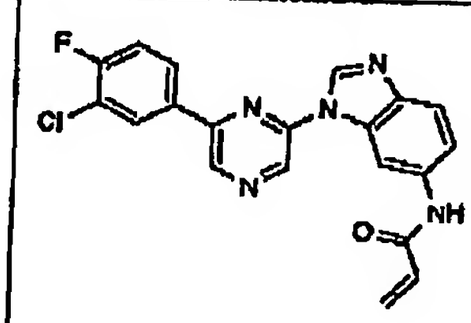
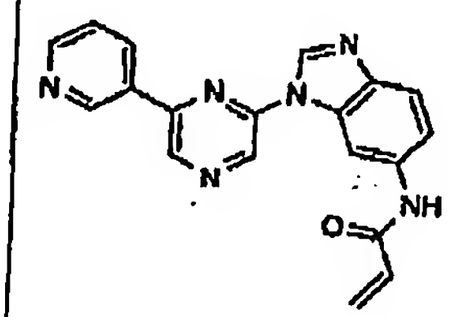
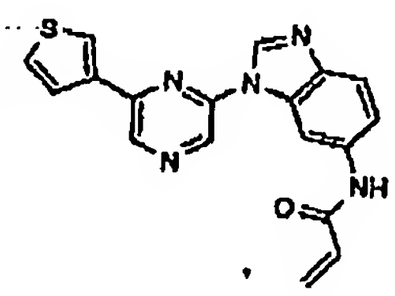
The following compounds were prepared using analogous procedures to those described above:

Compound	Structure	Data
N-[1-(6-Chloropyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		^{1}H-n.m.r. ($CDCl_3$) δ5.82 (dd, 1H, *J* = 9.8, 1.8Hz, =CH), 6.24-6.54 (m, 2H, $=CH_2$), 7.33 (dd, 1H, *J* = 8.8, 1.8Hz, ArH), 7.60 (br s, 1H, CONH), 7.80 (d, 1H, *J* = 8.4Hz, ArH), 8.58 (s, 2H, pyraz-H), 8.73 (br s, 1H, ArH), 8.94 (br s, 1H, ArH).
N-[1-[6-(4-Methylpiperazin-1-yl)pyrazin-2-yl]-1H-benzimidazol-6-yl]acrylamide		^{1}H-n.m.r. ($CDCl_3$) δ2.34 (s, 3H, NCH_3), 2.55 (t, 4H, *J* = 5.1Hz, CH_2), 3.74 (t, 4H, *J* = 5.1Hz, CH_2), 5.72 (dd, 1H, *J* = 9.0, 2.6Hz, CH), 6.25-6.48 (m, 2H, $=CH_2$), 7.14 (dd, 1H, *J* = 8.4, 2.2Hz, ArH), 7.68 (d, 1H, *J* = 8.6Hz, ArH), 8.04 (s, 1H, pyraz-H), 8.13 (s, 1H, pyraz-H), 8.38 (br s, 1H, CONH), 8.46 (s, 1H, ArH), 8.88 (br s, 1H, ArH).
N-[1-[6-(Diethylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl]acrylamide		^{1}H-n.m.r. ($CDCl_3$) δ1.24 (d, 6H, *J* = 7.0Hz, CH_3), 3.60 (q, 4H, *J* = 7.1Hz, CH_2), 5.72 (dd, 1H, *J* = 9.0, 2.7Hz, =CH), 6.25-6.49 (m, 2H, $=CH_2$), 7.20 (dd, 1H, *J* = 8.9, 2.0Hz, ArH), 7.71 (d, 1H, *J* = 8.4Hz, ArH), 7.91 (s, 1H, pyraz-H), 8.07 (s, 1H, pyraz-

		H), 8.33 (br s, 1H, CONH), 8.49 (s, 1H, ArH), 8.76 (br s, 1H, ArH).
N-[1-[6-(Methylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl]acrylamide		^{1}H-n.m.r. ($CDCl_3$) δ3.11 (d, 3H, J= 5.0Hz, CH_3), 4.9 (br s, 1H, NH), 5.78 (dd, 1H, J= 9.8, 2.2Hz, =CH), 6.23-6.51 (m, 2H, =CH_2), 7.15 (dd, 1H, J= 8.4, 2.2Hz, ArH), 7.63 (br s, 1H, CONH), 7.76 (d, 1H, J= 8.6Hz, ArH), 7.86 (s, 1H, pyraz-H), 8.13 (s, 1H, pyraz-H), 8.33 (s, 1H, ArH), 8.90 (s, 1H, ArH).
N-[1-[6-(Ethylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl]acrylamide		^{1}H-n.m.r. ($CDCl_3$/ d_4-MeOD) δ1.25 (t, 3H, J= 7.3Hz, CH_3), 3.42 (q, 2H, J= 7.3Hz, CH_2), 5.68 (dd, 1H, J= 7.8, 4.6Hz, =CH), 6.23-6.42 (m, 2H, =CH_2), 7.24 (dd, 1H, J= 8.6, 2.2Hz, ArH), 7.63 (d, 1H, J= 8.8Hz, ArH), 7.73 (s, 1H, pyraz-H), 7.97 (s, 1H, pyraz-H), 8.44 (s, 1H, ArH), 8.73 (br s, 1H, ArH).
N-[1-(6-piperidin-1-ylpyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		m/z (EI) 348 (M^+)

N-[1-(6-morpholin-4-ylpyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		*m/z* (EI) 350 (M^+)
N-[1-(6-pyrrolidin-1-ylpyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		*m/z* (EI) 334 (M^+)
N-(1-[6-(dimethylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl}acrylamide		*m/z* (EI) 308 (M^+)
N-(1-{6-[isopropyl(methyl)amino]pyrazin-2-yl}-1H-benzimidazol-6-yl)acrylamide		*m/z* (EI) 336 (M^+)
N-(1-[6-(Isopropylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl}acrylamide		^{1}H-n.m.r. ($CDCl_3$) δ1.32 (d, 6H, J = 6.2Hz, CH_3), 4.13-4.29 (m, 1H, CH), 4.75 (d, 1H, J = 7.8Hz, NH), 5.78 (dd, 1H, J = 9.8, 2.0Hz, =CH), 6.22-6.51 (m, 2H, $=CH_2$), 7.19 (dd, 1H, J = 8.6, 2.2Hz, ArH), 7.62 (br s, 1H, CONH), 7.76 (d, 1H, J = 8.8Hz, ArH), 7.82 (s, 1H, pyraz-H), 8.13 (s, 1H, pyraz-H), 8.50 (s,

		1H, ArH), 8.74 (br s, 1H, ArH).
N-[1-(6-{[(1S)-1-methylpropyl]amino}pyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		^{1}H-n.m.r. ($CDCl_3$) δ0.99 (t, 3H, J= 7.2Hz, CH_3), 1.27 (d, 3H, J= 6.4Hz, CH_3), 1.53-1.73 (m, 2H, CH_2), 3.95-4.09 (m, 1H, CH), 4.79 (d, 1H, J= 8.0Hz, NH), 5.76 (dd, 1H, J= 9.6, 2.0Hz, =CH), 6.23-6.50 (m, 2H, =CH_2), 7.21 (dd, 1H, J= 8.6, 2.2Hz, ArH), 7.74 (d, 1H, J= 8.8Hz, ArH), 7.82 (s, 1H, pyraz-H), 7.84 (br s, 1H, CONH), 8.11 (s, 1H, pyraz-H), 8.49 (s, 1H, ArH), 8.73 (br s, 1H, ArH).
N-[1-(6-{[(1*R*)-1-methylpropyl]amino}pyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		^{1}H-n.m.r. ($CDCl_3$) δ0.99 (t, 3H, J= 7.2Hz, CH_3), 1.27 (d, 3H, J= 6.4Hz, CH_3), 1.53-1.73 (m, 2H, CH_2), 3.95-4.08 (m, 1H, CH), 4.81 (d, 1H, J= 8.0Hz, NH), 5.75 (dd, 1H, J= 9.6, 2.0Hz, =CH), 6.23-6.50 (m, 2H, =CH_2), 7.22 (dd, 1H, J= 8.6, 2.2Hz, ArH), 7.73 (d, 1H, J= 8.8Hz, ArH), 7.81 (s, 1H, pyraz-H), 7.98 (br s, 1H, CONH), 8.10 (s, 1H, pyraz-H), 8.49 (s, 1H, ArH), 8.73 (br s, 1H, ArH).
N-[1-(6-Anilinopyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		^{1}H-n.m.r. ($CDCl_3$+d_4-MeOD) δ5.79 (dd, 1H, J= 9.0, 3.0Hz, =CH), 6.40 (1H, d, J= 9.0Hz, =CH(H)), 6.43 (1H, d, J= 3.0Hz, =CH(H)), 7.11-7.18 (m, 1H, ArH), 7.30-7.44 (m, 3H, ArH), 7.52-7.56 (m, 2H, ArH),

		7.75 (d, 1H, *J* = 8.8Hz, ArH), 8.20 (s, 1H, pyraz-H), 8.27 (s, 1H, pyraz-H), 8.56 (s, 1H, ArH), 8.79 (br s, 1H, ArH).
N-[1-(6-Phenylpyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		^{1}H-n.m.r. ($CDCl_3$) 85.70 (dd, 1H, *J* = 8.6, 2.2Hz, =CH), 6.22-6.46 (m, 2H, $=CH_2$), 7.29 (dd, 1H, *J* = 8.6, 1.4Hz, ArH), 7.47-7.57 (m, 3H, ArH), 7.68 (d, 1H, *J* = 8.8Hz, ArH), 8.12-8.16 (m, 2H, ArH), 8.65 (s, 1H, pyraz-H), 8.89 (s, 1H, pyraz-H), 8.91 (s, 1H, ArH), 8.97 (s, 1H, ArH).
N-[1-[6-(3-Chloro-4-fluorophenyl)pyrazin-2-yl]-1H-benzimidazol-6-yl]acrylamide		*m/z* (EI) 393, 395 (~3:1) (M^+)
N-[1-(6-Pyridin-3-ylpyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		*m/z* (EI) 342 (M^+)
N-[1-(6-Thien-3-ylpyrazin-2-yl)-1H-benzimidazol-6-yl]acrylamide		*m/z* (EI) 347 (M^+)

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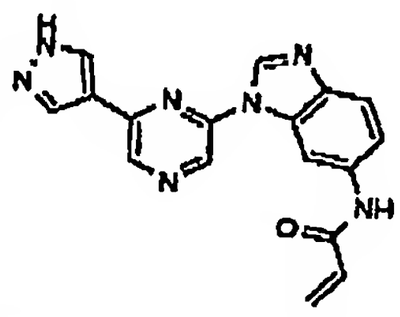
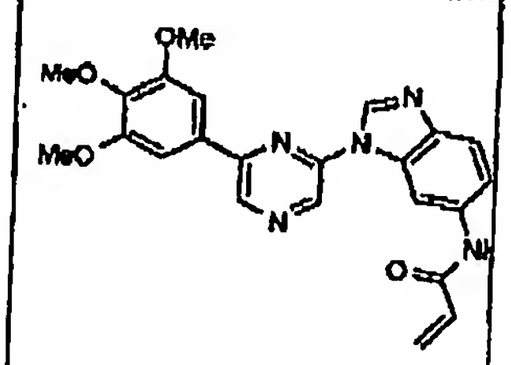
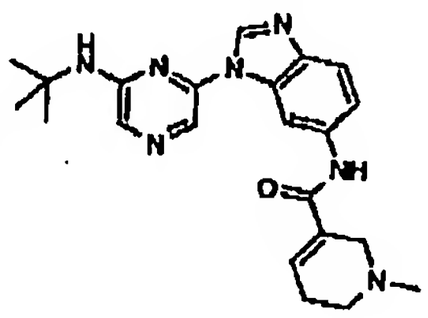
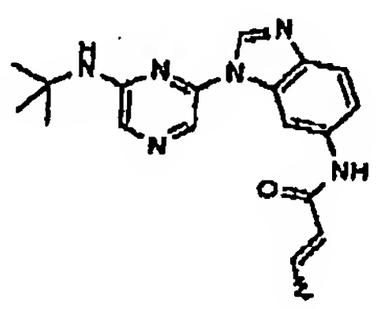
49.

N-[1-[6-(1H-Pyrazol-4-yl)pyrazin-2-yl]-1H-benzimidazol-6-yl]acrylamide		*m/z* (EI) 331 (M^+)
N-[1-[6-(3,4,5-trimethoxyphanyl)pyrazin-2-yl]-1H-benzimidazol-6-yl]acrylamide		*m/z* (EI) 431 (M^+)
1-Methyl-N-[1-[6-(t-butylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl]-1,2,5,6-tetrahydropyridine-3-carboxamide		*m/z* (EI) 405 (M^+)
N-[1-[6-(tert-butylamino)pyrazin-2-yl]-1H-benzimidazol-6-yl]but-2-enamide		*m/z* (EI) 350 (M^+)

SCREENING

Compound Dilution

For screening purposes, compounds were diluted in 96 well plates at a concentration of 20 µM. Plates were warmed at 37°C for 30 minutes before assay.

JAK Tyrosine Kinase Domain Production

JAK kinase domains were produced in the following manner:

JAK1

The kinase domain of human JAK1 was amplified from U937mRNA using the polymerase chain reaction with the following primers:

XHOI-J1 5'-CCG CTC GAG ACT GAA GTG GAC CCC ACA CAT-3'

J1-KPNI 5'-CGG GGT ACC TTA TTT TAA AAG TGC TTC AAA-3'

JAK1 PCR products were cloned into the pFastBac HTb expression vector (Gibco) via the Xho I and Kpn I sites. The JAK1 plasmid was then transformed into competent DH10Bac cells (Gibco), and the recombinant baculovirus produced prepared for transfection into Sf9 insect cells.

JAK2

The kinase domain of humanJAK2 was amplified from U937mRNA using the polymerase chain reaction with the following primers:

SALI-jk2 5'-ACG CGT CGA CGG TGC CTT TGA AGA CCG GGA T-3'

jk2-NOTI 5'-ATA GTT TAG CGG CCG CTC AGA ATG AAG GTC ATT T-3'

JAK2 PCR products were cloned into the pFastBac HTc expression vector (Gibco) via the Sal I and Not I sites. The JAK2 plasmid was then transformed into competent DH10Bac cells (Gibco), and the recombinant baculovirus produced prepared for transfection into Sf9 insect cells.

JAK3

The kinase domain of humanJAK3 was amplified from U937mRNA using the polymerase chain reaction with the following primers:

XHOI-J3 5'-CCG CTC GAG TAT GCC TGC CAA GAC CCC ACG-3'

J3-KPNI 5'-CGG GGT ACC CTA TGA AAA GGA CAG GGA GTG-3'

JAK3 PCR products were cloned into the pFastBac HTb expression vector (Gibco) via the Xho I and Kpn I sites. The JAK3 plasmid was then transformed into competent DH10Bac cells (Gibco), and the recombinant baculovirus produced prepared for transfection into Sf9 insect cells.

TYK2

The kinase domain of humanTYK2 was amplified from A549 mRNA using the polymerase chain reaction with the following primers:

HT2EK 5'-GGA GCA CTC GAG ATG GTA GCA CAC AAC CAG GTG-3'

ITY2.2R 5'-GGA GCA GGA ATT CCG GCG CTG CCG GTC AAA TCT GG-3'

TYK2 PCR products were cloned into pBlucBacHis2A (Invitrogen) via the EcoRI site. The recombinant TYK2 baculovirus produced was prepared for transfected into Sf9 insect cells.

Large Scale Production Of Kinase Domains

Baculovirus preparations from each of the JAK family members were infected into five litres of High Five cells (Invitrogen) grown in High Five serum free medium (Invitrogen) to a cell density of approximately 1-2 X 10^6 cells/ml. Cells are infected with virus at a MOI of 0.8-3.0. Cells were harvested and lysed. JAK kinase domains were purified by affinity chromatography on a Probond (Invitrogen) nickel chelate affinity column.

Assay Protocols

Kinase assays were performed either in a 96 well capture-based ELISA assay or in 384 well Optiplates (Packard) using an Alphascreen Protein Tyrosine Kinase kit. In either casse using approximately 1.5 µg of affinity purified PTK domain in the presence of 50mM HEPES, pH 7.5, 10mM $MgCl_2$, 150mM NaCl and 10µM-1mM ATP. The biotinylated substrate biotin-EGPWLEEEEEAYGWMDF-NH_2 (final concentration 5µM) was used as substrate. In the ELISA assay tyrosine phosphorylation was quantitated

following transfer to an avidin coated ELISA plate using peroxidase-linked anti-phospho-tyrosine antibody PY20. In the Alphascreen assay, Alphascreen phosphotyrosine acceptor beads followed by streptavidin donor beads were added under subdued light. The ELISA plates were read on a BMG Fluorostar, the Alphascreen plates were read on a Packard Fusion *Alpha*. Inhibitors were added to the assays fifteen minutes prior to the addition of ATP. Inhibitors were added in aqueous DMSO, with DMSO concentrations never exceeding 1%.

Results

The activity of selected compounds is shown in Table 3. Compounds that exhibited a capacity to inhibit 50% of JAK activity at a concentration of 20μM (measured under standard conditions, see Methods), are designated as "+".

Table 3

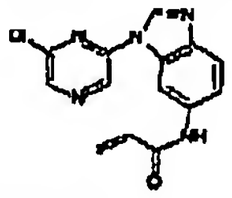
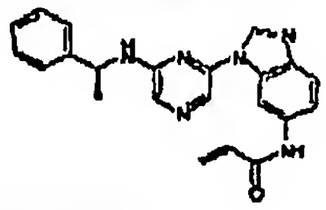
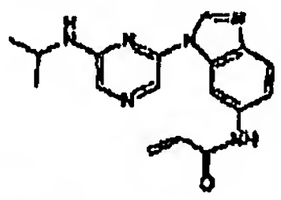
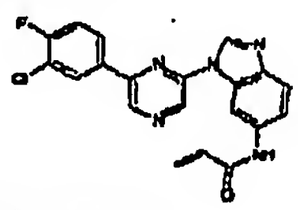
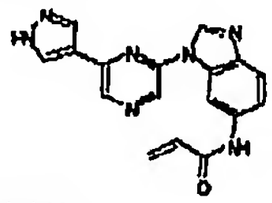
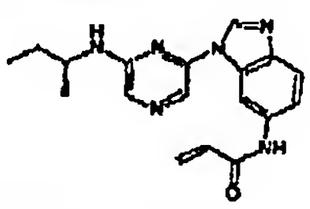
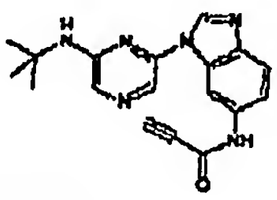
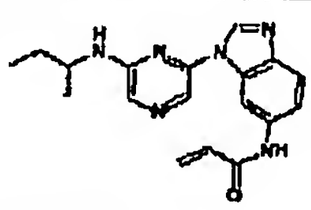
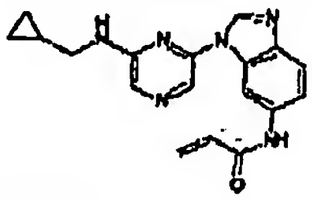
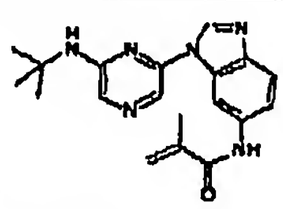
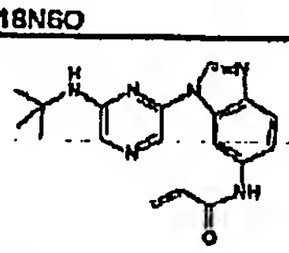
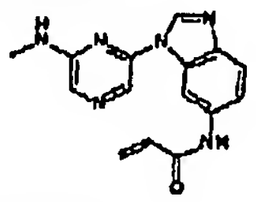
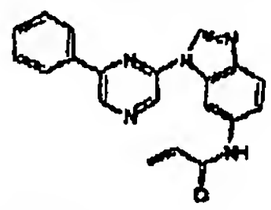
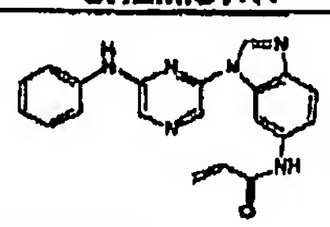
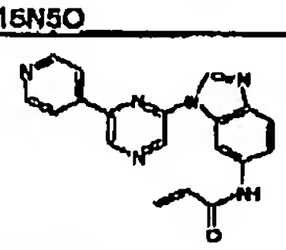
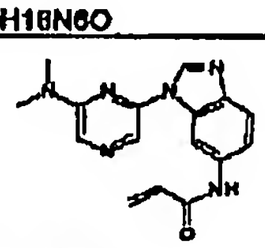
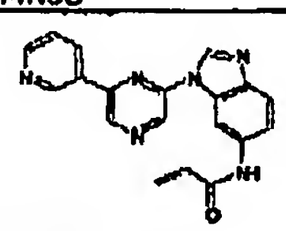
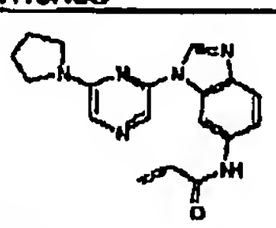
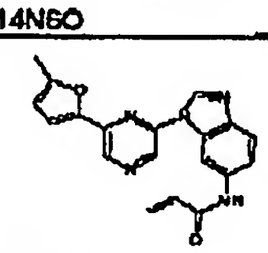
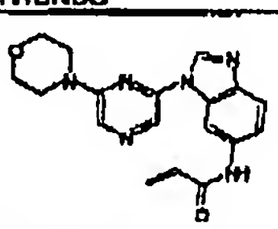
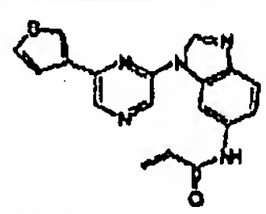
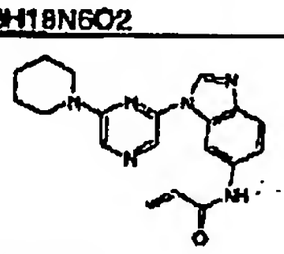
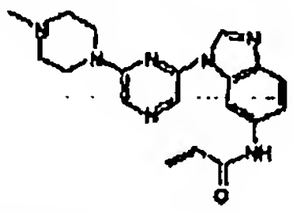
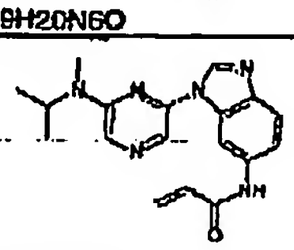
CHEMISTRY	Jak2	Jak3	CHEMISTRY	Jak2	Jak3
 C14H10ClN5O	-	+	 C22H20N6O	+	+
 C17H18N8O	-	+	 C20H13ClFN5O	-	+
 C17H13N7O	-	+	 C18H20N6O	-	+
 C18H18N8O	-	+	 C18H20N6O	-	+
 C18H18N6O	-	+	 C19H22N6O	-	+
 C18H20N6O	-	+	 C15H14N6O	-	+

Table 3 (cont.)

CHEMISTRY	Jak2	Jak3	CHEMISTRY	Jak2	Jak3
 C20H15N5O	-	+	 C20H16N6O	-	+
 C19H14N6O	-	+	 C16H16N6O	-	+
 C19H14N6O	-	+	 C18H18N6O	-	+
 C19H15N5O2	-	+	 C18H18N6O2	-	+
 C18H13N5OS	-	+	 C19H20N6O	-	+
 C19H21N7O	-	+	 C18H20N6O	-	+

Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

All publications mentioned in this specification are herein incorporated by reference. Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed in Australia or elsewhere before the priority date of each claim of this application.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

Dated: 12 January 2004

Cytopia Pty Ltd

Patent Attorneys for the Applicant:

BLAKE DAWSON WALDRON PATENT SERVICES

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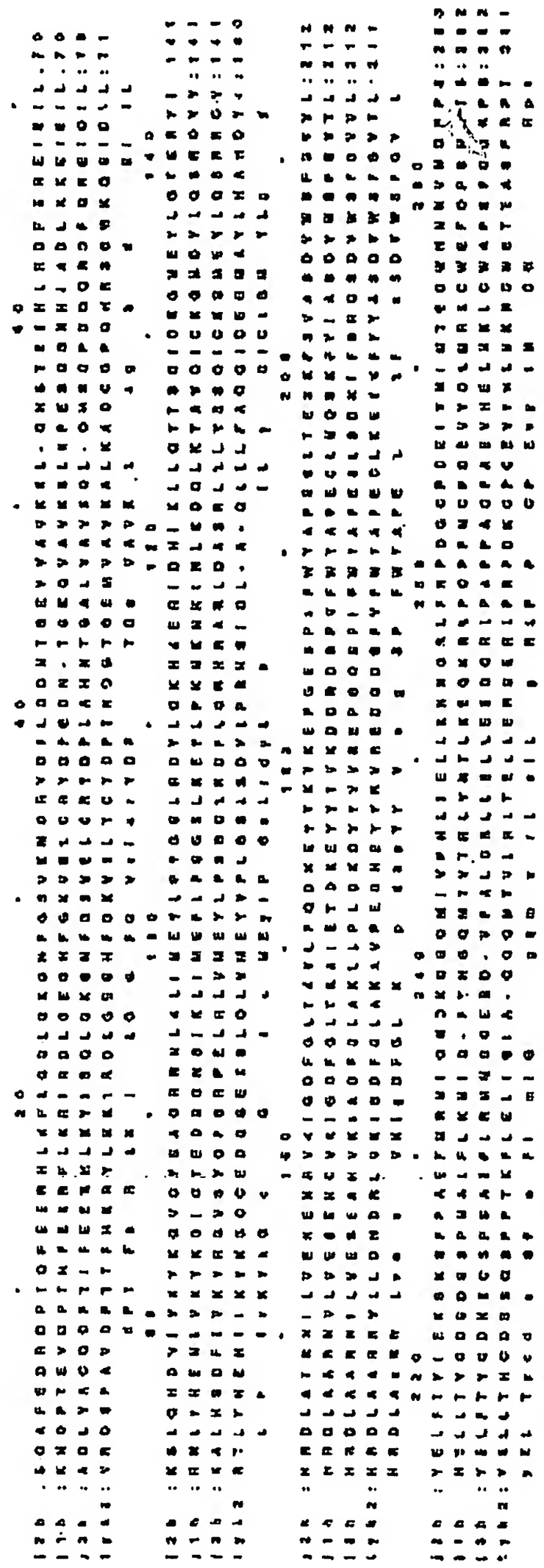

Figure 1

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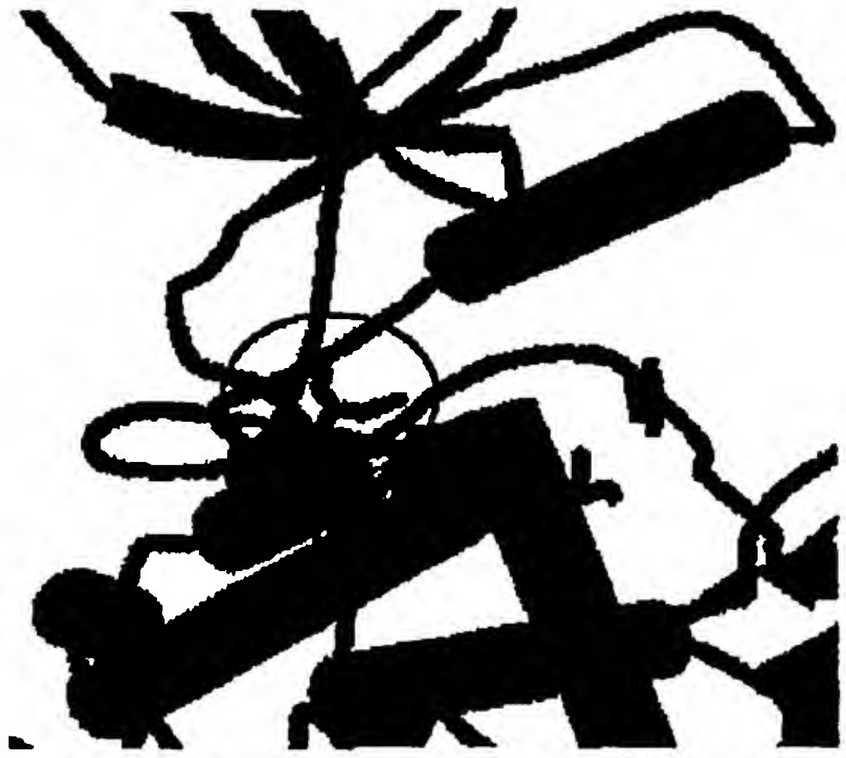

Figure 2